# THE ASCORBIC ACID CONTENT OF CHICK BLOOD

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ONE FIGURE

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Early investigations of chick rations did not show a need for supplementing the chick rations of that era with vitamin C in order to obtain normal health and the desired rate of growth. Hart, Halpin and Steenbock ('22) stated that the antiscorbutic vitamin requirements of growing chicks could be met by the quantities found in a cereal grain and skimmed milk. Emmett and Peacock ('22) reported that the vitamin C requirements of the chick appeared to be much less than those for vitamins A and B. After working with a potent tomato extract these authors ('23) concluded that during the early stages of the chick's growth, vitamin C was not essential, and questioned its value in the diets of laying hens.

Mitchell, Kendall and Card ('23) investigated vitamin C free diets supplemented with tomato juice and reported that chicks were not susceptible to scurvy. Plimmer, Rosedale and Raymond ('23) conducted a 14-month experiment with chickens and reported "it may still be concluded that chickens, pigeons and other birds never seem to require the addition of C-vitamin to their food."

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Shorten and Ray ('21) fed fowls a basal ration of rice, supplemented with a variety of sun-dried vegetables, and found no apparent effect on the birds.

Following a study on the effect of adding vitamins to chick rations Knox and Lamb ('24) stated, "Our data do not demonstrate any need of chicks for the antiscorbutic vitamin, although the tomato juice improved the ration which had been heated to destroy vitamin C."

This review of the literature revealed data principally concerned with chick rations and feeding conditions in vogue 12 to 16 years ago. In the mean time the composition of chick rations has been significantly modified. Also the present day commercial conditions demand a higher rate of chick growth than in the past. These changes in chick rations and rate of growth creates renewed interest in the role of vitamin C in chick nutrition. Furthermore, because of the abundant supply of vitamin C found in the liver, kidney, adrenals, brain and other organs, it is a generally accepted fact that vitamin C is of importance in the normal metabolism of the chick. Exact knowledge concerning the part played by vitamin C in the metabolism of the chick is still unavailable and the literature fails to reveal any information concerning the amounts of vitamin C in the blood plasma of the normal growing chick. Accordingly the present investigation was undertaken to accumulate data in this connection.

## EXPERIMENTAL

Rhode Island Red baby chicks were obtained from the same source as those reported in previous investigations (Holmes and co-workers, '31, '34), conducted by this laboratory. These November hatched chicks were produced by vigorous, disease-free stock. They were housed from hatching to 12 weeks of age in metal battery brooders maintained under the same conditions as described in a previous publication (Holmes, Tripp and Campbell, '36).

Three pens of thirty chicks each received an all-mash ration during the entire 12-week experimental period. The all-mash ration was composed of yellow corn meal 33⅓%, wheat

bran 15%, wheat flour middlings 15.86%, ground oat groats 12%, dried skimmed milk 8%, alfalfa leaf meal 5%, meat scraps 5%, fish meal 5%, oyster shell meal 1%, iodized salt 0.5%, dicalcium phosphate 0.5%, manganese sulphate 0.0125%. The all-mash ration fed to the chicks of pen 1 was supplemented with 0.063% of a fortified sardine oil which had a potency of 400 U.S.P. XI vitamin D units per gram. The ration of pen 2 contained 0.125% of the same oil, and the ration fed to the chicks in pen 3 was supplemented with 0.063% of a second lot of fortified sardine oil with a potency of 400 vitamin D units per gram.

The rations were assayed for their vitamin C content according to the method described by Bessey and King ('33) and modified by Mack and Tressler ('37). Five grams of the ration was ground with 30 gm. of acid-washed white sand and 25 cc. of the acid mixture (2% $HPO_3$, 5% $H_2SO_4$). The mass was centrifuged, decanted, and washed with three 7 cc. portions of the acid mixture. The final volume was made up to 50 cc. A 5-cc. portion of the extract was transferred to an Erlenmeyer flask and 5 cc. of the acid mixture added. This solution was then titrated with 2, 6 dichlorophenolindophenol until a faint pink end point was reached. The dye solution was prepared and standardized in a manner similar to that described by Tripp, Satterfield and Holmes ('37). The amount of ascorbic acid per 100 gm. of ration was not in excess of 0.94 mg. for ration no. 1, 1.02 mg. for ration no. 2 and 1.00 mg. for ration no. 3.

When the chicks were 8 and 12 weeks old, typical cockerels and pullets were selected from each of the three pens. The chicks were fasted for about 1 hour before killing. The ascorbic acid content of the blood plasma was determined by the method of Satterfield, Perlzweig and Dann ('38). Twenty cubic centimeters of blood were collected from the jugular vein into flint glass bottles of 50 cc. capacity, previously coated with potassium oxalate (40 mg. to 20 cc. blood). Ten milligrams of sodium cyanide were also added to each 20 cc. blood sample as an antioxidant. The blood samples were immediately transferred to 15 cc. centrifuge tubes and centrifuged

for 20 minutes at 1000 r.p.m. Five cubic centimeters of the straw colored blood plasma was pipetted from the tube and diluted with 5 cc. of distilled water in a 50 cc. Erlenmeyer flask. Ten cubic centimeters of 4% metaphosphoric acid was added to this mixture. The precipitated proteins were filtered off and 5 cc. of the clear filtrate was pipetted into a clean

TABLE 1

*Ascorbic acid content of the blood plasma of 8-week-old chicks*

| PEN NO. | MONTH TESTED | CHICK | | | ASCORBIC ACID PER 100 CC. PLASMA |
|---|---|---|---|---|---|
| | | Sex | Weight | Condition | |
| | | | gm. | | |
| 1 | January | M | 1106 | Good | 2.100 |
| 1 | January | M | 1049 | Good | 1.971 |
| 1 | January | F | 709 | Good | 1.795 |
| 1 | January | F | 992 | Good | 2.044 |
| | | | Average | | 1.978 |
| 2 | January | M | 1021 | Good | 2.205 |
| 2 | January | M | 1134 | Good | 2.190 |
| 2 | January | F | 851 | Good | 2.146 |
| 2 | January | F | 794 | Good | 1.854 |
| | | | Average | | 2.099 |
| 3 | January | M | 992 | Good | 2.146 |
| 3 | January | M | 794 | Good | 1.562 |
| 3 | January | F | 794 | Good | 2.219 |
| 3 | January | F | 794 | Good | 2.234 |
| | | | Average | | 2.040 |
| | | | Average (12 birds) | | 2.039 |

50 cc. Erlenmeyer flask. The dichlorophenolindophenol solution was added from a micro-burette until a faint pink end point was reached.

The amount of ascorbic acid per 100 cc. of blood plasma was calculated according to the following formula:

$$\frac{(\text{cc. dye used})\ (\text{ascorbic acid equiv. of dye})}{\text{cc. filtrate used}} \times \frac{(\text{final dilution})}{\text{cc. plasma used}} = \text{mg. ascorbic acid per 100 cc. plasma}$$

It will be noted from table 1 that the ascorbic acid content of the blood plasma of the 8-week-old birds from pens 1, 2 and 3 was 1.978, 2.099 and 2.040 mg., respectively, per 100 cc.

of plasma. The average value for the twelve 8-week-old chicks was 2.039 mg. of ascorbic acid per 100 cc. of plasma. The two cockerels in pen 1 averaged 2.036 mg. of ascorbic acid per 100 cc. of blood plasma while the average for the two pullets was 1.920 mg. per 100 cc. of plasma. In pen 2 the average for the two cockerels was 2.198 mg. ascorbic acid per 100 cc. of plasma while the two pullets averaged 2.000 mg.

TABLE 2

*Ascorbic acid content of the blood plasma of 12-week-old chicks*

| PEN NO. | MONTH TESTED | CHICK | | | ASCORBIC ACID PER 100 CC. PLASMA |
|---|---|---|---|---|---|
| | | Sex | Weight | Condition | |
| | | | *gm.* | | |
| 1 | February | M | 1362 | Good | 1.727 |
| 1 | February | F | 1589 | Good | 2.192 |
| 1 | February | F | 1249 | Good | 2.425 |
| 1 | February | F | 1476 | Good | 2.159 |
| 1 | February | F | 1334 | Good | 1.462 |
| | | | Average | | 1.993 |
| 2 | February | M | 1362 | Good | 1.511 |
| 2 | February | M | 1476 | Good | 1.993 |
| 2 | February | F | 1390 | Good | 2.309 |
| 2 | February | F | 1504 | Good | 2.342 |
| 2 | February | F | 1419 | Good | 2.375 |
| | | | Average | | 2.106 |
| 3 | February | M | 1731 | Slipped tendon | 1.910 |
| 3 | February | F | 1362 | Good | 2.093 |
| 3 | February | F | 1362 | Good | 1.889 |
| 3 | February | F | 1220 | Good | 2.093 |
| 3 | February | F | 1334 | Good | 2.325 |
| | | | Average | | 2.062 |
| | | | Average (15 birds) | | 2.054 |

The blood plasma of the cockerels of pen 3 contained 1.854 mg. ascorbic acid per 100 cc. while the pullets averaged 2.227 mg. It is interesting to note that the birds containing the maximum and minimum amounts of ascorbic acid in their blood plasma were from pen 3.

The data for the 12-week-old chicks are included in table 2. The ascorbic acid content of the blood plasma of the chicks of pen 1 averaged 1.993 mg., of pen 2, 2.106 mg., of pen 3,

2.062 mg. per 100 cc. of plasma. The average ascorbic acid content for the fifteen 12-week-old chicks was 2.054 mg. per 100 cc. of plasma. The ascorbic acid content of the blood plasma of the cockerels in each of the three pens was lower than that for the pullets for the respective pens but these data are not sufficient to permit any general conclusions concerning the relation of sex to the ascorbic acid content of chick blood.

Data concerning the rate of growth of the experimental chicks, the amount of mash consumed and the extent to which the mash was utilized is of interest for evaluating the results of the ascorbic acid assays of chick blood. These data are reported in figure 1 and table 3.

Inspection of figure 1 will reveal that the rate of growth was the same for all three groups of chicks for the entire 12-week period. The rate of weekly increase in mash consumption was not quite as consistent as the weekly increase in body weight. The cause of the lower mash consumption during the fourth week is not known but twenty other experimental pens of chick of the same age that were in the laboratory at the same time showed a similar decrease in mash consumption.

The 'growth' reported in table 3 represents the difference between the initial weight and the weight of the chick at 8 or 12 weeks of age. The values reported for mash consumption represent the total per bird consumption for the 8 or 12-week period. The nutritive efficiency of the mashes has been computed by determining the amount of each mash required for each 100 gm. gain in chick body weight during the first 8 or 12 weeks of life.

For all practical purposes the nutritive efficiency for the three mashes for the 8-week period is identical, although the values obtained indicate that the growth promoting value of mashes 1, 2 and 3 decreased slightly in the order listed. The nutritive efficiency of the three mashes for the 12-week period was highest for mash no. 2 and decreased for mash no. 3 and mash no. 1. Considered on a practical basis, the growth promoting qualities of mash no. 2 are appreciably superior to

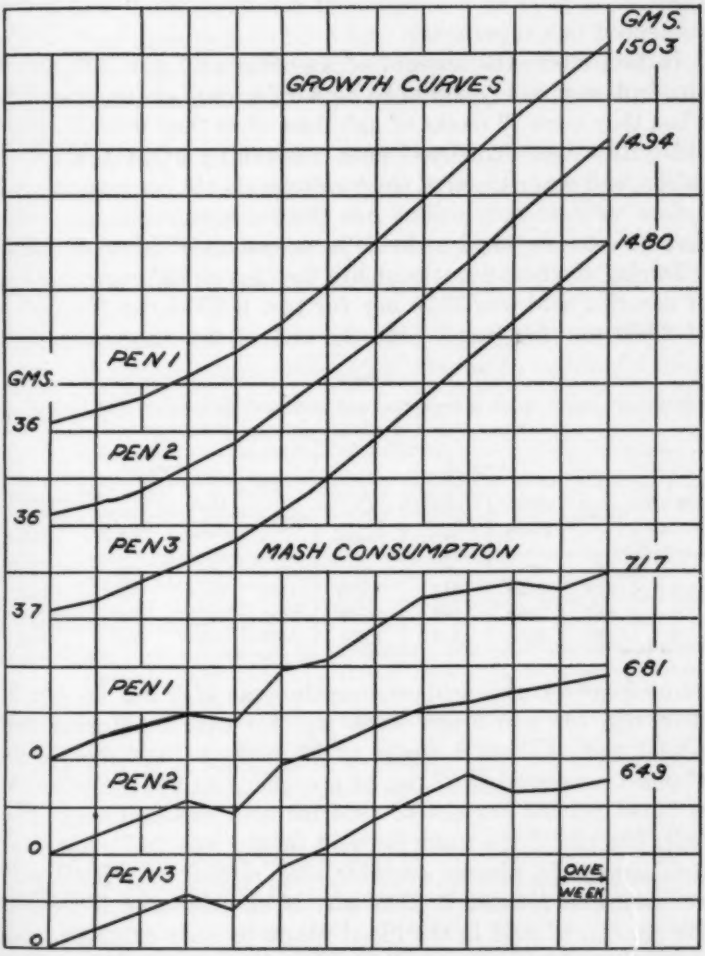


Fig. 1 Rate of growth and weekly mash consumption of Rhode Island Red chicks used for determination of the ascorbic acid content of chick blood.

those of mash no. 1. Possibly the larger amount of fortified sardine oil used as a supplement for mash no. 2 may have influenced this superiority.

In this study the amount of ascorbic acid per 100 cc. of blood plasma was found to be higher for each group of chicks when they were 12 weeks of age than when they were 8 weeks old. However, additional data concerning other breeds of chicks, and other types of chick rations should be accumulated before definite conclusions are drawn concerning the relation between age and ascorbic acid content of blood plasma.

During the first 8 weeks of life the 'per chick' consumption of ascorbic acid was 22.95 mg. for pen 1, 23.37 mg. for pen 2 of 23.60 mg. for pen 3. At the end of the 12-week period

TABLE 3

*Relation of growth, mash consumption and utilization to ascorbic acid content of blood plasma of rapidly growing chicks*

| PEN NO. | 8 WEEKS | | | | 12 WEEKS | | | |
|---|---|---|---|---|---|---|---|---|
| | Growth | Mash consumed | Amount of mash per 100 gm. gain | Ascorbic acid per 100 cc. plasma | Growth | Mash consumed | Amount of mash per 100 gm. gain | Ascorbic acid per 100 cc. plasma |
| | *gm.* | *gm.* | *gm.* | *mg.* | *gm.* | *gm.* | *gm.* | *mg.* |
| 1 | 870 | 2442 | 281 | 1.978 | 1466 | 5140 | 350 | 1.993 |
| 2 | 843 | 2389 | 284 | 2.099 | 1457 | 4910 | 337 | 2.106 |
| 3 | 829 | 2360 | 285 | 2.040 | 1416 | 4880 | 345 | 2.062 |

the average ascorbic acid consumption was 48.32 mg. for pen 1, 50.08 mg. for pen 2 and 48.80 mg. for pen 3. During the 4-week period from 8 weeks to 12 weeks of age the chicks of pen 1 consumed 25.37 mg. of ascorbic acid but the increase in blood plasma content of ascorbic acid was 0.015 mg. per cent, for pen 2 the ascorbic acid intake was 26.71 mg. and the increase in plasma ascorbic acid content was 0.007 mg. per cent and for pen 3, 25.20 mg. of ascorbic acid increased the amount of acid in the blood plasm by only 0.022 mg. per cent. Such data seem to indicate that the ascorbic acid content of the blood plasma is approximately 2.0 mg. per 100 gm. of plasma for rapidly growing chicks during the 8 weeks to 12 weeks period.

SUMMARY

Methods for determining the ascorbic acid content of blood plasma and chick rations have been described.

Data concerning the vitamin C (ascorbic acid) content of blood plasma for 8- and 12-week-old chicks have been accumulated.

The ascorbic acid content of the blood plasma of rapidly growing 8-week-old chicks averaged 2.039 mg. per cent. The amount varied between 1.562 and 2.234 mg. per cent.

The ascorbic acid content of the blood plasma of vigorous 12-week-old chicks averaged 2.054 mg. per cent, varying from 1.462 to 2.425 mg. per cent.

A comparison of the ascorbic acid blood plasma values of cockerels and pullets indicates that there is no consistent relationship between sexes.

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# DOES FAT IN THE DIET AFFECT THE THYROID?[1]

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The Mellanbys ('21), in connection with their work on rickets in puppies, observed that when fat, particularly butterfat, was present in the diet the thyroid glands were enlarged and hyperplastic. When on the other hand the fat was cod liver oil, the glands were small and normal in appearance. They reported a fivefold increase in thyroid weight for butter over cod liver oil. In a second series in which the fat intake was increased from 10 to 20 gm. per day, the effect on the thyroid was still further increased. Likewise McCarrison ('22) reported that when pigeons were fed a diet of millet supplemented by butter or oleic acid, the size of the thyroid was increased. The glands of his birds on basal diet alone weighed 537 mg., with 13% butter 675 mg., with 3.2% oleic acid 909 mg., but with cod liver oil only 93 mg. In another experiment McCarrison ('32) found that seventeen pigeons receiving 4.5 gm. per day of butter, together with a diet of mixed grains, suffered an average thyroid enlargement of more than two times.

The above work has been frequently cited, particularly by Marine ('35) as evidence that the amount of fat, especially unsaturated fat, in the diet exerts an influence on the size and state of the thyroid gland. In neither of the investigations mentioned was any attempt made to explain the very small glands obtained when the fat was cod liver oil which contains

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a much higher percentage of unsaturated acids than does butter fat. Nor was there any reference to an attempt to determine the iodine content of the diets, or to rule out the possibility that the observed effects could be attributed in whole or in part to iodine deficiency. Freudenberger and Clausen ('35) also report that rats fed 1% of cod liver oil had significantly smaller thyroids than those not receiving it. Cod liver oil is known to contain a measurable, and indeed if fed in any quantity, a physiologically significant amount of iodine (Lunde, '29; Holmes and Remington, '35), and in a form which appears to be quite readily metabolized. If the diets used in the experiments of Mellanby and McCarrison happened to be deficient in iodine, this deficiency would work to bring about enlargement and hyperplasia of the thyroid, and which could conceivably be still further enlarged by replacing any considerable proportion of the caloric value of the diet with a non-iodine-bearing fat, but the enlargement would be prevented or at least retarded if the fat were cod liver oil. From this point of view the pigeons to which McCarrison fed cod liver oil were normal, those receiving no fat very markedly goitrous, and those receiving 13% butterfat only slightly more so. This explanation does not account for the behavior of oleic acid, however. In McCarrison's second experiment the amount of butter fed, if it could all be metabolized, would more than satisfy the basal energy requirement of the birds (Brody and Procter, '32), and hence the question of diminished iodine intake cannot be dismissed.

If fat in the diet tends to produce goiter, three possible modes of action suggest themselves:

a. An interference with the utilization of the iodine of the diet for the synthesis of the thyroid colloid, which might be due either to the ability of fatty acids to take up iodine and combine it into stable compounds, or to some use of iodide or other iodine compounds in the intermediary fat metabolism, and in the course of which the iodine is excreted.

b. An increased drain on the colloid secretion of the gland so great as to lead to partial or complete exhaustion, with

resultant hyperemia and hypertrophy. Such an effect might be expected if the thyroid hormone has a specific function in fat metabolism.

c. An effect on the gland independent, primarily, of iodine supply or iodine metabolism, such an effect for example as might result if the fat contained some substance which exerted a specific toxic action on the secreting epithelial cells of the thyroid.

In either of the first two cases iodine deficiency in the diet could be expected to enhance the effect, but in the third case it would be independent of iodine supply and would indicate some action on the gland not associated with the storage of iodine.

Levine, Remington and von Kolnitz ('33 b) have shown that in rats fed diets varying only as to iodine content, there is a definite and striking relationship between the iodine intake and the size and degree of hyperplasia of the thyroid. Remington ('37) has recently improved the basal diet so as to be able to secure practically normal growth and development, and without lessening the degree of goiter produced. Remington and Levine ('36) have also been able to show and express by curves a relationship between the weight of the gland and its iodine content, as well as degree of edema, on iodine deficient diets which were varied in a number of ways. No evidence has as yet been found in this laboratory that the characteristic picture described by Levine, Remington and von Kolnitz ('33 a) ever occurs except as a result of iodine deficiency.

In view of the foregoing it seemed desirable to apply our technic to an investigation of the effect of adding both saturated and unsaturated fat to an iodine deficient diet, as well as to the same diet made more nearly adequate by the addition of sodium iodide. If the metabolism of fat is concerned in any way in the utilization of iodine by the thyroid for the synthesis of the iodine-containing colloid, or in the discharge of this colloid from the gland, we can expect significant differences to be found in the weight, dry matter and iodine content of

the glands of rats fed fat with an iodine deficient diet as compared with those which received none or only the amount unavoidably present in the basal diet. Feeding of fat with a diet that is better supplied with iodine will determine whether it has a goitrogenic action independent of iodine metabolism.

## EXPERIMENTAL

The method differs only in minor details from that described in my earlier papers. With the former diet 'GP' the experimental period was limited to the time the rats could be kept alive and in fairly good condition, and nearly all work was done on a 35-day period. My new diet 342 [2] removes this restriction and one may use any length of time desired. Significant results can be obtained in 5 weeks, but the thyroid condition becomes progressively more aggravated up to 10 or 12 weeks at least. The fat was added at levels of 2.5, 5.0 and 10.0% to the previously prepared basal diet. As an example of a highly saturated fat hydrogenated cottonseed oil [3] was chosen, and refined cottonseed oil [4] as an unsaturated fat. The basal diet itself contained 4.02% of fat from yellow corn meal and dried liver, so that when 10% of other fat was added the total amounted to 13.6% of the diet or 30% of the calories. Each group consisted of ten animals weighing at the start between 50 and 60 gm., and equally divided as to sex. The results on the low iodine series are presented in part A of the table.

The thyroid glands of normal rats of the same weight as these at the end of the experiment weigh 8 mg. per 100 gm. body weight, contain 33% of dry matter and 0.200% of iodine, dry basis (Remington, Remington and Welch, '37). All animals of the low iodine series, regardless of the amount of fat in the diet, show a strikingly uniform enlargement of approximately 2.5 times normal and the differences among groups are not statistically significant. The dry matter is in all cases

| | | | |
|---|---|---|---|
| [2] Wheat gluten | 18 | Calcium carbonate | 1 |
| Yellow corn meal | 78 | Sodium chloride | 1 |
| Dried pig liver | 2 | | |

[3] Crisco.

[4] Wesson Oil.

in good accord with the curve of Remington and Levine, no value varying more than 0.6% from that predicted from the weight of the glands. The iodine content of the various groups varied from 3 to 7% of that of normal glands, and considering the difficulties of estimating such minute amounts of iodine, are in good agreement with one another. We cannot conclude from any of the criteria used in our work (size of gland, dry matter and iodine content) that the fat added to the diet has exerted any influence whatsoever on the development of goiter

TABLE 1

*Effect of dietary fat on the thyroid gland*

| DIET NO. | DESCRIPTION | GAIN IN WEIGHT | THYROID GLANDS | | | |
|---|---|---|---|---|---|---|
| | | | Weight | Weight per 100 gm. body weight | Dry matter | Iodine dry basis |
| A. Diet deficient in iodine. Feeding period 8 weeks. Ten rats in each group | | | | | | |
| | | *gm.* | *mg.* | *mg.* | % | % |
| 342 | Basal | 126.0 | 37.0 | 20.5±1.03 | 23.40 | 0.014 |
| 350 | 2.5% Wesson Oil | 121.9 | 35.9 | 19.8±1.10 | 24.35 | 0.006 |
| 351 | 5.0% Wesson Oil | 112.5 | 31.2 | 18.1±0.68 | 23.42 | 0.008 |
| 352 | 10.0% Wesson Oil | 87.3 | 28.5 | 19.8±0.51 | 23.74 | 0.015 |
| 353 | 2.5% Crisco | 107.2 | 30.9 | 19.6±0.93 | 23.61 | 0.011 |
| 354 | 5.0% Crisco | 106.5 | 31.6 | 19.9±1.01 | 22.93 | 0.010 |
| 355 | 10.0% Crisco | 88.9 | 28.8 | 20.1±0.93 | 23.20 | 0.009 |
| B. Diet with added iodide. Feeding period 6 weeks. Eight rats in each group | | | | | | |
| 342I | Basal | 118.7 | 19.8 | 12.0±0.42 | 30.9 | 0.110 |
| 364 | 10.0% Wesson Oil | 78.2 | 16.9 | 13.5±0.35 | 33.8 | 0.157 |
| 365 | 10.0% Crisco | 89.7 | 16.7 | 12.5±0.52 | 33.2 | 0.145 |

in iodine deficiency. It might be argued that the iodine deficiency is so severe that the glands are exhausted, so to speak; that is to say, have reached such a degree of hyperplasia that it is impossible for fat further to augment the effect. This seems hardly probable, since the degree of enlargement attained in this series is not as great as has been observed in many earlier experiments, this seeming to vary with different lots of diet, and perhaps with the season of the year. It is not at all unusual for groups of rats on this diet to show average enlargements of three to four times normal. It hardly

seems likely that these rats, with thyroids enlarged to 2.5 times normal, have reached a stage of hyperplasia where they would be resistant to a goitrogenic influence in fat if such existed.

For the diet more adequate as to iodine, an alcoholic solution of sodium iodide was evaporated on yellow corn meal, and enough of this iodized meal used in diet 342 so that each 10 gm. of completed diet contained 5 γ of iodine. This diet is called 342-I and differs only in iodine content from diet 342. The average food consumption during the experimental period is 10 to 12 gm. per rat per day, so that each rat received 5 to 6 γ of iodine per day. Using this diet as a basis, fat was added in a manner similar to the preceding, except that in this case both saturated and unsaturated fats were fed at the 10% level only. In this case the diets were fed for 6 weeks, and eight animals, equally divided as to sex, were placed in each group (part B of table).

The glands of all rats, on higher iodine intake, with and without fat, are about 1.25 times as heavy as our normals, but again the differences between groups are not significant. The dry matter content is normal for all groups, in fact that of the fat-fed rats is higher, indicating better storage of colloid. Iodine storage is not decreased in fat-fed animals. While histologically these glands are not as well filled with colloid as those of rats on colony ration, they cannot be classed as goitrous. The results clearly negate any influence of dietary fat, saturated or unsaturated, up to 30% of the caloric intake, on the thyroid gland.

This amount of fat is not abnormally high, being exceeded in many human dietaries. Difficulties were encountered in getting rats to grow well with more than 10% of added fat in the diet. Although they make excellent growth, and can be kept alive and in apparent good condition almost indefinitely on diet 342, the addition of fat, either with or without iodine, has a marked deleterious effect, as shown in the table. In addition to retarded growth, fully half of the rats on the diets highest in fat showed loss of hair, amounting in some cases

to almost complete denudation, but no symptoms were observed that could be correlated with any specific vitamin deficiency. Before the effects of abnormally high fat intakes can be studied, the margin of safety of the basal diet as to known vitamins and inorganic salts will have to be determined, and steps made to correct any near-deficiencies.

## SUMMARY

Addition of saturated or unsaturated fat up to 13.6% of the weight or 30% of the caloric value of an iodine deficient diet, does not aggravate the hyperplasia of the thyroid produced by iodine deficiency.

When sodium iodide is added to the diet so as to yield 5 γ of iodine to each 10 gm. of diet, the addition of fat is still without effect on the thyroid.

The experiments refute the theory that there is any specific function of the thyroid gland in the metabolism of fat.

Adding fat to a diet consisting of wheat gluten 18, dried pig liver 2, yellow corn meal 78, calcium carbonate 1 and sodium chloride 1, retards growth and produces non-specific symptoms of deficiency.

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# THE AVAILABILITY OF IRON IN VARIOUS FOODS[1]

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Research within the past few years indicates that the influence of different foods upon the regeneration of hemoglobin in nutritional anemia varies widely. The bioassay technic which has been used extensively to determine the hematopoietic properties of foods measures the availability of iron for hemoglobin synthesis in young rats rendered anemic by prolonged feeding of an exclusive milk diet, provided copper is amply supplied.

Another method for determining the availability of iron in foodstuffs has been proposed by Elvehjem and his colleagues. From an extensive series of investigations (Elvehjem et al., '33, '34; Sherman, Elvehjem and Hart, '34; Sherman, Hart and Elvehjem, '34; Kohler et al., '36), they concluded that the ionizable, or non-hematin, iron which can be determined with the reagent $\alpha, \alpha'$-dipyridyl is identical with the available iron in a food. Surprisingly low values were reported by them for the ionizable iron content of green leafy vegetables previously considered excellent sources of iron in the human diet. Shackleton and McCance ('36) found considerably higher percentages of ionizable iron in greens. In contrast to the findings of Elvehjem and co-workers discrepancies between the values for the ionizable iron in certain foods and the iron available for hemoglobin formation were reported recently by Smith and Otis ('37 b).

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The purpose of this investigation was to determine the hematopoietic value of certain leafy vegetables which are important sources of iron in southern dietaries, namely, collards, turnip greens, mustard, tendergreen, kale, spinach, and leaf and head lettuce. Blackeyed peas, which are rich in iron, were also tested. Both the ionizable iron and the iron available for the regeneration of hemoglobin were to be determined and any correlations noted.

## PROCEDURE

The greens were grown under similar conditions and harvested at approximately the same degree of maturity. After being thoroughly washed they were dried at 60° to 65°C. and ground in a ball mill until fine enough to pass through a 40-mesh sieve. The dried blackeyed peas purchased locally were too hard to break in the mill, consequently they were cooked in a minimum of distilled water, mashed, dried and then ground. There was sufficient dried material for the entire experiment in the final homogeneous mixture of each food. In order to study the effect of cooking on the availability of iron, collards and one lot of turnip greens were canned by the cold-pack process and the leaves and liquid fed in proportional amounts. A second lot of turnip greens was cooked and the leaves and liquid were canned separately.

The iron content of the food materials was determined by the modification of Elvehjem's method developed by Farrar ('35).

Attempts to determine the ionizable iron content of the dried foods by the $\alpha, \alpha'$-dipyridyl method used by Elvehjem (Elvehjem, Hart and Sherman, '33) were not successful. The pigments in the dried materials masked the red color of the final solutions to such an extent that comparisons with the standards were impossible. Neither precipitation with lead acetate (Kohler, Elvehjem and Hart, '36) nor bleaching with $SO_2$ (Hill, '30) was effective in removing the interfering pigments. Another source of error in this method was occasioned by the adsorption of the colored complex on the plant tissue.

The modification of Hill's method ('30) developed by Shackleton and McCance ('36) was used in this work to determine ionizable iron. In this procedure pigment interference is precluded by the use of a comparator and the adsorption of the colored complex is prevented by postponing color development in the extract with $\alpha, \alpha'$-dipyridyl until after the removal of the solid material by filtration. For our determinations a block comparator of the Walpole type with tubes graduated at 10 cc. was used. Thirteen standards were prepared in 10 cc. dilutions containing 0.0500, 0.0475, 0.0450, . . . . 0.0200 mg. of iron, respectively. Iron-free sodium hydrosulfite was used as the reducing agent. Blanks were run for each determination but none were as high as 0.000125 mg. of iron per cubic centimeter.

The experimental rats were reared by mothers fed a stock diet of one-third whole milk powder and two-thirds ground whole wheat plus small portions of lean beef and string beans. The animal technic was similar in many respects to that of Elvehjem and Kemmerer ('31), the principal difference being that the mothers were fed the stock diet in the cage with their young. Preliminary work had shown that feeding them in separate cages did not appreciably shorten the depletion time of the young.

At 21 days of age the rats were weaned and placed on the depletion diet of fresh whole milk. Special care was taken to prevent contamination of the milk with iron. It was milked directly into an enamel pail, strained into glass jars, and fed in glass dishes. All of the receptacles with which the milk came in contact were carefully washed and then thoroughly rinsed in distilled water.

After 5 to 7 weeks on the milk diet, the hemoglobin concentration in the rats had fallen below 3.3 gm. per 100 cc. of blood. Hemoglobin measurements were made by the Newcomer method. The depletion level ranged from 3.3 gm. to below 2.5 gm., the average being 2.8 gm., which was used as the basis for computing the gains during the experimental period. At this low level it was believed that the iron reserves

of the body had been exhausted. Smith and Otis ('37 c) found that the hemoglobin response of anemic rats to additions of iron and copper was much less when copper was fed during the depletion period and concluded that the giving of copper during this time was necessary for the complete utilization of the iron stores of the body. Rose and Hubbell ('38) concluded that preliminary copper feeding caused such slight increases in hemoglobin that the administration of copper during the depletion period was not necessary. Hence, it is debatable whether copper is essential for the exhaustion of the iron reserves, especially when the hemoglobin has been reduced to a very low level.

The animals were placed in individual Hendryx cages at depletion and started on the test supplements. The distribution of rats to the various supplements was made as evenly as possible in respect to sex, age, weight and hemoglobin level. The supplements were fed daily on the basis of a 6-day week; in the case of the dried foods a week's supply was weighed out from which approximately equal amounts were fed daily. Each daily portion was moistened continually with small volumes of milk until it was completely consumed, after which the milk was fed ad libitum. Constant care was taken that the entire supplement was eaten; animals which had not consumed the amount allotted for the experimental period were discarded. Weekly records of weight and hemoglobin level were kept from the second to the end of the 6-week test period.

Representative animals were continued on the milk diet after depletion as negative controls. Not one of them showed any significant rise in hemoglobin, and of the forty-one controls only seven survived until the end of the 6 weeks.

Each animal receiving an iron-containing supplement was given daily 0.05 mg. of copper, as copper sulfate, in order that hemoglobin synthesis might proceed uncomplicated by a deficiency of this element. Manganese sulfate, supplying 0.04 mg. of manganese daily, was given to each of the positive controls which received iron at various levels in the form of ferric chloride.

## RESULTS AND DISCUSSION

The results of the analyses of the dried foods for total and ionizable iron are shown in table 1. Comparison of these values for ionizable iron with those obtained by other investigators revealed certain similarities and differences. The range of 60 to 70% of ionizable iron in raw and cooked green leafy vegetables reported by Shackleton and McCance ('36) is rather similar to ours of 60 to 95% in dried greens. In comparison, the values of 19 to 20% and of 21 to 28% for raw spinach and lettuce, respectively, found by Kohler, Elvehjem and Hart ('36) are very low. Shackleton and McCance re-

TABLE 1

*The total and ionizable iron content of certain dried foods*

| MATERIAL | TOTAL IRON | IONIZABLE IRON IN PER CENT OF TOTAL IRON |
|---|---|---|
| | *mg./gm.* | % |
| Spinach | 0.372 | 70 |
| Mustard | 0.331 | 95 |
| Kale | 0.308 | 75 |
| Leaf lettuce | 0.245 | 60 |
| Turnip greens | 0.225 | 75 |
| Tendergreen | 0.200 | 60 |
| Head lettuce | 0.170 | 60 |
| Blackeyed peas | 0.147 | 55 |
| Collards | 0.101 | 60 |

ported 75% of ionizable iron in raw spinach and 63% in raw lettuce; our values for dried spinach and leaf or head lettuce are 68% and 60%, respectively. Our determinations showed 55% of the iron to be ionizable in blackeyed peas which had been cooked and dried, a lower value than the 70 to 80% in fresh and dried blue peas reported by Shackleton and McCance or than the 81% in dried peas reported by Smith and Otis ('37 b).

The average ages and weights at depletion of the animals on the various supplements and their gains in weight during the experimental period are shown in table 2. No correlation has been noted between any of these factors and the rise in

hemoglobin. Despite the severity of the depletion, the addition of the iron-containing supplements to the milk diet immediately caused large gains in weight.

Iron, as ferric chloride, was fed at three levels, 0.3, 0.4, and 0.5 mg. daily. The respective gains in hemoglobin of 8.3, 9.8, and 9.6 gm. raised the hemoglobin levels of these animals to 11.1, 12.6, and 12.4 gm. In normal stock animals of the same age the hemoglobin level was 13.2 to 13.8 gm. Apparently complete regeneration of hemoglobin was not possible on these levels of iron within the limited time of the experimental period. Other laboratories have reported complete regeneration in 6 weeks on 0.3 mg. of iron, but in several of our animals receiving this quantity of iron it required 10 weeks for the hemoglobin to rise to normal. It is probable that the severity of the depletion in our animals necessitated the greater length of time for the return to the normal level. Since 0.4 mg. of iron induced as great a rise of hemoglobin as 0.5 mg., it was obvious that neither of these levels was suitable for measuring availability. The gain on 0.3 mg. of iron was significantly lower than on the higher levels, hence differences in response due to differences in availability should have been apparent at this level. For this reason, and also because it had been used by other workers, 0.3 mg. of iron was chosen as the test level for feeding the materials under investigation.

The values reported for gains in hemoglobin are averages of the data for both male and female rats. Smith and Otis ('37 a), having found the hemoglobin response of the females greater than that of the males, advised considering the data for each sex separately. In our work consistently greater gains were not made by the females. For example, the average gains in hemoglobin of fourteen males and thirteen females on supplements of turnip greens containing 0.3 mg. of total iron were identical, namely, 6.3 gm. The thirty-one males and twenty-one females receiving 0.3 mg. of iron, as ferric chloride, responded with equal average gains of 8.3 gm. Although the hemoglobin gains for each sex were not always identical on the other supplements, there was no evident rela-

TABLE 2

*The average ages and weights of rats at depletion and the gains in body weight during the 6-week experimental period on various supplements*

| SUPPLEMENT | NUMBER OF RATS | | AGE AT DEPLETION | | WEIGHT AT DEPLETION | | GAIN IN WEIGHT | |
|---|---|---|---|---|---|---|---|---|
| | Males | Females | Males | Females | Males | Females | Males | Females |
| **Rats receiving ferric chloride supplements** | | | | | | | | |
| | | | *days* | *days* | *gm.* | *gm.* | *gm.* | *gm.* |
| Iron, 0.3 mg. | 31 | 28 | 65 | 63 | 80 | 81 | 116 | 89 |
| Iron, 0.4 mg. | 11 | 11 | 71 | 72 | 71 | 77 | 126 | 83 |
| Iron, 0.5 mg. | 5 | 4 | 67 | 67 | 85 | 98 | 121 | 58 |
| **Rats receiving food supplements containing 0.3 mg. of total iron** | | | | | | | | |
| Mustard | 5 | 5 | 55 | 59 | 99 | 90 | 145 | 96 |
| Turnip greens | 14 | 15 | 65 | 68 | 83 | 83 | 118 | 83 |
| Kale | 5 | 4 | 65 | 73 | 77 | 78 | 117 | 83 |
| Spinach | 9 | 10 | 63 | 70 | 83 | 88 | 102 | 75 |
| Collards | 5 | 6 | 60 | 68 | 87 | 90 | 130 | 85 |
| Tendergreen | 5 | 3 | 62 | 67 | 87 | 87 | 114 | 94 |
| Head lettuce | 4 | 6 | 54 | 58 | 98 | 87 | 155 | 106 |
| Leaf lettuce | 4 | 4 | 57 | 55 | 87 | 86 | 135 | 99 |
| Blackeyed peas | 4 | 6 | 65 | 65 | 88 | 80 | 140 | 85 |
| Canned collards: leaves and liquid | 4 | 6 | 61 | 66 | 93 | 89 | 141 | 88 |
| Canned turnip greens: leaves and liquid | 5 | 7 | 66 | 68 | 85 | 95 | 131 | 94 |
| Canned turnip greens: leaves | 4 | 5 | 66 | 63 | 86 | 77 | 120 | 85 |

tion between sex and hemoglobin response. In evaluating the results, therefore, males and females were not differentiated.

In our experience the precision of the bioassay technic for determining availability of iron was not as great as might be desired. The rather wide range of variation in the hemoglobin gains on a given supplement, shown by the values for the standard deviation of the mean (S.D.), made it impossible for small differences to be considered significant. In comparing differences in hemoglobin response, it must be remembered that the rate of hemoglobin regeneration is not arithmetically proportional to the iron available, since lower levels of iron produce greater gains in proportion to the amount fed than do higher levels. Therefore, to determine the percentage of available iron in a food, it would be necessary either to have reference curves of response to graded supplements of iron in completely available form (suggested by Smith and Otis, '37 b), or to find the minimum quantity of the food in question which would produce a rise in hemoglobin equivalent to that of the positive controls. The former procedure is being developed for subsequent work; the latter proved to be impractical in certain instances because the animals were not able to consume the increased amount of food supplement.

To test whether bulk per se in the food supplements might have had any purely mechanical influence on the absorption of iron from the intestinal tract, Celluflour, a material possessing bulk but not food value, was added to the milk-iron diet of the positive controls receiving 0.3 mg. of iron. As shown in table 3, no significant difference was found between the hemoglobin response to the milk-iron diet and to the same diet plus 0.5 gm. or 0.75 gm. Celluflour daily.

The average gains in hemoglobin of anemic rats receiving sufficient food materials to furnish 0.3 mg. of total iron daily are shown in table 3. In no instance were these gains as great as that of the positive controls receiving 0.3 mg. of iron. Blackeyed peas caused the greatest gain in hemoglobin, 7.8 gm., which was almost equal to that of the positive controls. The smallest gains were 4.2 and 4.0 gm. produced by tender-

green and collards, respectively. The results show that the various dried foods fall into the following descending order in regard to the availability of their iron for the regeneration of hemoglobin: blackeyed peas and spinach, turnip greens and kale, collards and mustard, head lettuce and lastly, tendergreen and leaf lettuce.

TABLE 3

*The hemoglobin regeneration of anemic rats given various levels of iron, as ferric chloride, and food supplements containing 0.3 mg. of total iron*

| SUPPLEMENT | NUMBER OF RATS | IRON IN SUPPLEMENT | | GAIN IN HEMOGLOBIN | ±S.D.[2] |
|---|---|---|---|---|---|
| | | Total | Ionizable | | |
| | | *mg./day* | *mg./day* | *gm./100 cc.* | |
| $FeCl_3$ | 59 | 0.3 | 0.3 | 8.3 | 0.21 |
| $FeCl_3$ | 22 | 0.4 | 0.4 | 9.8 | 0.25 |
| $FeCl_3$ | 9 | 0.5 | 0.5 | 9.6 | 0.31 |
| $FeCl_3$ + 0.5 gm. Celluflour | 6 | 0.3 | 0.3 | 8.4 | 0.55 |
| $FeCl_3$ + 0.75 gm. Celluflour | 11 | 0.3 | 0.3 | 8.5 | 0.33 |
| Mustard | 10 | 0.3 | 0.29 | 5.2 | 0.34 |
| Turnip greens | 29 | 0.3 | 0.23 | 6.3 | 0.30 |
| Kale | 9 | 0.3 | 0.23 | 6.3 | 0.55 |
| Spinach | 19 | 0.3 | 0.21 | 7.4 | 0.41 |
| Collards | 11 | 0.3 | 0.18 | 5.6 | 0.30 |
| Tendergreen | 8 | 0.3 | 0.18 | 4.2 | 0.33 |
| Head lettuce | 10 | 0.3 | 0.18 | 4.7 | 0.51 |
| Leaf lettuce | 8 | 0.3 | 0.18 | 4.0 | 0.45 |
| Blackeyed peas | 10 | 0.3 | 0.17 | 7.8 | 0.41 |
| Canned collards: leaves and liquid | 10 | 0.3 | ...[1] | 6.6 | 0.38 |
| Canned turnip greens: leaves and liquid | 12 | 0.3 | ...[1] | 7.1 | 0.44 |
| Canned turnip greens: leaves | 9 | 0.3 | ...[1] | 5.4 | 0.47 |

[1] Not determined.

[2] Standard deviation of the mean.

Canned collards with both leaves and liquid present in proportional amounts caused a rise in hemoglobin of 6.6 gm., greater than that of 5.6 gm. on dried collards. The gain of 7.1 gm. on canned turnip greens, with both leaves and liquid, was greater than that of 6.3 gm. on dried turnip greens. The iron in these canned products was, therefore, better utilized

than that in the dried greens. The hemoglobin rise on canned turnip greens alone was only 5.4 gm., lower even than that on the dried greens. It proved impossible to feed the canned turnip green liquid as such because the animals refused to eat it. It seemed probable, however, that the superiority of the combination of canned leaves and liquid to either the dried greens or the canned leaves alone was due to a higher percentage of available iron in the liquid.

The ionizable iron contents of the food supplements providing 0.3 mg. of total iron are shown in table 3. There was no evidence of any relation between the amount of ionizable

TABLE 4

*The hemoglobin regeneration of anemic rats given 0.3 mg. of ionizable iron*

| SUPPLEMENT | NUMBER OF RATS | IONIZABLE IRON | | GAIN IN HEMOGLOBIN | ±S.D.[1] |
|---|---|---|---|---|---|
| | | In food supplement | As $FeCl_3$ | | |
| | | *mg.* | *mg.* | *gm./100 cc.* | |
| Turnip greens | 6 | 0.3 | | 6.7 | 0.52 |
| Kale | 5 | 0.3 | | 6.6 | 0.94 |
| Spinach | 8 | 0.28 | | 7.2 | 0.22 |
| Mustard | 10 | 0.285 | 0.015 | 6.7 | 0.36 |
| Turnip greens | 9 | 0.225 | 0.075 | 6.8 | 0.32 |
| Kale | 9 | 0.225 | 0.075 | 7.0 | 0.34 |
| Spinach | 10 | 0.210 | 0.090 | 8.0 | 0.39 |
| Collards | 6 | 0.180 | 0.120 | 6.8 | 0.36 |
| Tendergreen | 10 | 0.180 | 0.120 | 7.2 | 0.32 |
| Blackeyed peas | 10 | 0.165 | 0.135 | 8.1 | 0.26 |

[1] Standard deviation of the mean.

iron in them and the gain in hemoglobin. For example, blackeyed peas which contained only 0.17 mg. of ionizable iron induced a greater rise in hemoglobin than mustard with 0.29 mg. The effects of collards, tendergreen, head lettuce and leaf lettuce were dissimilar, even though these supplements provided identical amounts of ionizable iron.

Further investigation of the correlation between ionizable and available iron was pursued by feeding 0.3 mg. of ionizable iron in the food supplement (table 4). If the ionizable iron of a food is the same as its available iron, the hemoglobin response in animals receiving 0.3 mg. of ionizable iron in the

food supplement should be as great as that of the positive controls receiving 0.3 mg. of iron. Animals fed sufficient quantities of spinach, kale, and turnip greens to furnish 0.3 mg. of ionizable iron showed hemoglobin gains of 7.2, 6.7, and 6.6 gm., respectively, not one of which was as great as the gain of the positive controls. Since it was difficult and in several cases impossible to feed enough supplement to furnish 0.3 mg. of ionizable iron, a modified procedure for feeding at this level was followed. To each food supplement containing 0.3 mg. of total iron, sufficient ferric chloride was added to make the ionizable iron equivalent to 0.3 mg. The amounts of iron added as well as the gains in hemoglobin produced by these combinations are given in table 4. On only two of these supplements, blackeyed peas and spinach, did the animals attain the hemoglobin level of the positive controls. The other supplements produced various smaller gains. From the data in tables 3 and 4 it seems conclusive that in these foods the ionizable iron and the iron available for hemoglobin synthesis were not identical.

Smith and Otis ('37 b) suggested that the discrepancies found between the ionizable and available iron contents of certain foods might have been due to their relatively high roughage content. In our work the negative effect of Cellu-flour additions to the milk-iron diet seemed to show that bulk per se does not influence the utilization of iron. The reason for the superiority of the canned greens with their liquid to either the canned leaves or the dried greens is as yet undetermined. Whatever the factor or factors influencing the utilization of iron, the value for the ionizable iron content of a food does not appear to be a reliable measure of the iron which is available to the body for hemoglobin production.

## CONCLUSIONS

The regeneration of hemoglobin in response to certain foods was measured in nutritionally anemic rats. Also, the ionizable iron in these foods was determined chemically by the $\alpha, \alpha'$-dipyridyl method.

A greater hemoglobin gain was obtained with the canned leaves plus the cooking liquid of turnip greens and collards than with the dried forms of these greens. The iron of the canned turnip green leaves was less available than that of the dried greens.

The foods studied fall into the following descending order in respect to the availability of their iron for the regeneration of hemoglobin: blackeyed peas and spinach, turnip greens and kale, collards and mustard, head lettuce and finally, tendergreen and leaf lettuce. In these foods the ionizable iron and the iron available for hemoglobin synthesis were not identical.

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# A QUANTITATIVE STUDY, BY MEANS OF SPECTROGRAPHIC ANALYSIS, OF COPPER IN NUTRITION [1]

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TWO FIGURES

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Gerlachs' ('34) success in the application of spectrum analysis to biological materials suggested the possibility of using this method in nutrition balance studies. Homologous pairs of lines obtained from the spectrum produced by burning the material between carbon electrodes, first reported by Gerlach in 1925, seemed applicable to a study of the need of the human organism for some of the rarer elements. By this procedure errors due to contamination should be greatly diminished, since the use of reagents is negligible. Furthermore, it seemed possible that this method, which is sensitive enough to detect the mere presence of an element, could be used with suitable standards to determine copper quantitatively.

Although copper metabolism studies with adults are reported by Thompsett ('34) and Chou and Adolph ('35), Daniels and Wright ('34) were the only authors using subjects of preschool age. With the adults, the food and excreta were analyzed by the carbamate method; with the children, Elvehjem and Lindow's ('29) modified Biazzo method which McFarlane ('32) and Ansbacher, Remington and Culp ('31) found to be less sensitive. This may in part account for the lower retentions in the former study.

[1] The data of this study are taken from a dissertation submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in nutrition, Child Welfare Research Station, State University of Iowa.

In the present study the retentions were determined by the spectrographic method. The copper content of the food, feces, urine, distilled water and acid-alcohol used as a preservative, being determined by reading the length of the copper and reference lines of their spectrograms and comparing these differences with those produced by standard solutions.

## MATERIALS AND METHODS

Three normal boys (F.V., G.E. and J.E.) ranging in age from 3 to 6 years, served as subjects in six successive double metabolism periods as described by Daniels and co-workers ('35).

During the entire study only two major changes were made in the original diet (diet I) which consisted of a whole grain bread and cereal, butter, green beans, carrots, potatoes, bananas, apple sauce, prunes, eggs, ground lean beef and milk. Each child also received specified amounts of Viosterol, cod liver oil and orange juice daily. The first change (diet II) consisted in substituting white bread and Cream of Wheat for the whole grain bread and cereal, tomatoes for green beans, and decreasing the amount of orange juice by one-half. The second change (diet III) consisted in a reduction in the amount of meat and egg and an increase in the quantity of fruit, vegetables and butter. Whenever possible, the necessary adjustments for the increased caloric needs of the given child were made by slight changes in sugar.

The method employed in obtaining the aliquots of food, feces and urine also is given by Daniels and Wright ('34) with the exception that, in the present study, the urines were made up to volume with water redistilled through a block tin condenser. To avoid contamination only well-aged glassware was used for the storage of both food and excreta, and all of the porcelain and glassware was given the preliminary treatment recommended by Elvehjem and Lindow ('29). Bright aluminum utensils were used in the preparation of the cooked foods.

The duplicate 4 gm. samples of dried food, first used, were increased to 6 gm. when it was found that the smaller samples gave spectrographic lines which were less distinct and more difficult to read. Two grams of the dried feces and 500 ml. of urine were used throughout the study. Both the food and feces were ashed for 12 hours at dull red heat in a muffle furnace, whereas the urine was ashed in the air with the aid of two Fischer burners. Five hundred milliliter samples from each carboy used of distilled water, as well as acid-alcohol, were evaporated and ignited prior to dilution.

Each of the ashed samples was dissolved in 3 ml. of hydrochloric acid and made up to a volume of 10 ml. in calibrated Pyrex volumetric flasks with redistilled water.

The copper standards were made by diluting a solution of copper sulphate, the copper content of which had been determined by electroplating on platinum (Popoff, '27).

When the preliminary work of the study was undertaken, it was found that the spectrographic quality of graphite was contaminated with a variable amount of copper. A special spectrographic quality,[2] however, was found to be free from copper and consequently was used for the analysis of copper. All analyses were made in duplicate.

The 1½ inch electrodes used for spectrographic analysis were prepared in a manner similar to that reported by Popoff, Waldbauer and McCann ('32). The electrodes were scraped before using, subsequently arced for 30 seconds (Lamb, '35), cooled and wrapped in cellophane until used. Four drops of the solution to be tested were placed in the well of the electrode from the same pipette used in calibrating the volumetric flasks. These electrodes, previous to arcing, were baked for not less than 12 hours in an oven at about 300°C.

In addition to the procedure employed by Popoff, Waldbauer and McCann ('32) and Waldbauer and Gantz ('33), a rotating sector disk was placed before the slit of the spectrograph.

[2] The author is grateful to the Graduate College for the funds with which to purchase the special spectrographic quality graphite necessary for the quantitative determination of copper.

The sector disk, which was maintained at about 20 r.p.m., has been shown by Twyman and Hitchen ('31) to produce 'wedge spectra' in which the length of the line is a measure of its intensity from graded standards.

Following the improved method of Gerlach ('25) for the use of an internal standard in quantitative spectrographic work, bands at 3889 Angstroms, which always gave maximum length of line in the obtained spectra, were used. As an aid in reading the length of the copper lines, 3247.5 and 3274, and the bands at 3889 Angstroms, the spectrogram was placed on the glass surface of an x-ray viewing box. Calipers, graduated in tenths of a millimeter, were used in obtaining these measurements. When the differences between the length of the copper line and the reference bands checked to within 0.2 mm. they were plotted for each concentration (fig. 1). The concentration of the copper present in the unknowns (fig. 2) was obtained by comparing the difference between the copper line and the internal standard with those produced by the standard solution.

Since the persistent lines 3247.5 and 3274 Angstroms were the only copper lines appearing on all the spectrograms, they were used in reading the concentration of the copper in the samples of food and excreta. Spectrograms made from the ash of both the distilled water and acid-alcohol aliquots showed 3274 Angstroms indistinctly; therefore, the copper concentration was based upon the 3247.5 line. The standard copper solution spectra, however, were read for both 3247.5 and 3274 Angstroms whenever possible. When the concentration of the copper of the solutions of food and excreta were read from these curves, the milligrams of copper agreed to within the third decimal place for the two lines.

In the first attempts to test the accuracy of the spectrographic method a known amount of copper, 0.005 mg., was added to the electrode of samples prior to arcing and the recovery calculated. The range of from 100 to 200% was thought to be due to the difference in the ions present, or to the total ion concentration. Twyman and Hitchen ('31) and Duffendack, Wolf and Smith ('35) showed that the addition of other

ions to a known solution altered the length of the spectra lines of certain elements. More recently Cholak ('35) reports that standards used in testing for lead in urines should have the same ion concentration as the unknowns, although variations greater than 50% were required to produce significant changes in results. Therefore, the standard solutions used in the

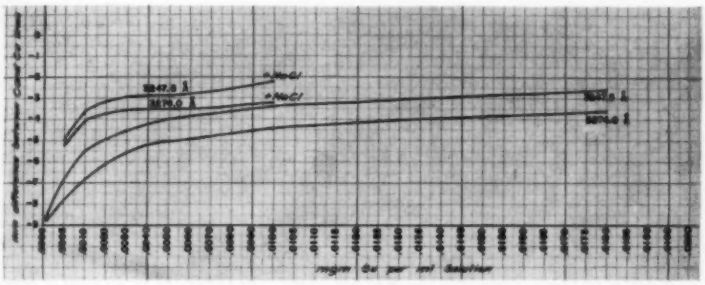

Fig. 1 Copper concentration in milligrams plotted for each tenth of a millimeter difference between the length of the copper and reference lines in spectra produced by standard solution, with and without the addition of sodium chloride.

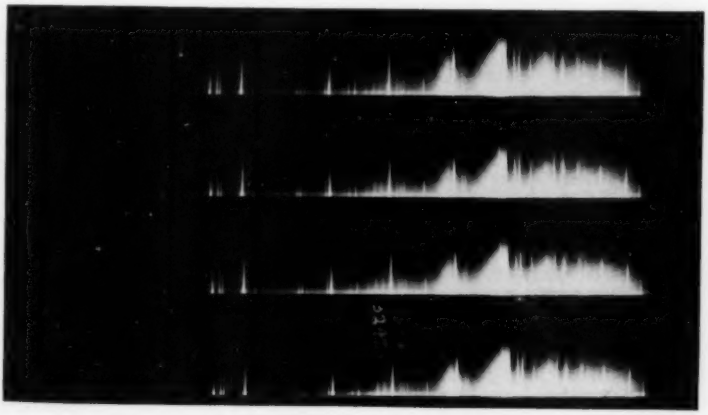

Fig. 2 Spectra of urine ash with copper line 3247.5 Angstroms marked and the reference bands indicated by (r). 1 and 2 represents duplicate samples of F.V.'s ashed urine, while 3 and 4 are samples of G.E.'s ashed urine for the same metabolism periods.

present study were made to approximate the urine samples being tested by adding 4 drops of a standard solution of urine salts [3] to the electrode in which a standard copper solution had been dried. When these were arced the intensity of the sodium flame so nearly resembled that produced by the arcing of the urine electrodes that a standard solution of pure sodium chloride was substituted for the urine salts. That these two salt solutions had a similar effect upon the two most persistent copper lines is shown by the fact that the addition of 1 drop of either solution gave comparable readings (table 1).

TABLE 1

*Comparison of the effects of NaCl and urine salts upon the difference between the lengths of the two copper lines when these salts were added to an electrode containing 0.005 mg. of copper*

| SATURATED NaCl SOLUTION | Cu 3247.5A | Cu 3274A | DIFFERENCE | URINE SALTS | Cu 3247.4A | Cu 3274A | DIFFERENCE |
|---|---|---|---|---|---|---|---|
| *drops* | *mm.* | *mm.* | *mm.* | *drops* | *mm.* | *mm.* | *mm.* |
| 0 | 9.4 | 8.3 | 1.1 | 0 | 9.4 | 8.3 | 1.1 |
| 1 | 9.4 | 8.3 | 1.1 | 1 | 10.5 | 9.3 | 1.2 |
| 2 | 9.8 | 9.0 | 0.8 | 2 | 10.7 | 9.4 | 1.3 |
| 3 | 11.1 | 10.5 | 0.6 | 4 | 10.0 | 9.1 | 0.9 |
| 4 | 11.2 | 10.8 | 0.4 | 5 | 10.6 | 9.9 | 0.7 |
| 5 | 11.5 | 11.2 | 0.3 | 6 | 10.7 | 10.2 | 0.5 |

However, 5 drops of the urine salts or 3 drops of the saturated sodium chloride solution produced significant differences, namely 0.7 and 0.6 mm. This ion effect may also offer an explanation for the results obtained by Boyd and De ('33) when they employed sodium chloride to "increase the volume of their residue" when testing for lead and manganese.

The curve used in reading the concentration of copper in the spectrograms made from the solution of the samples to be tested, was governed by the difference between the lengths

[3] Inorganic solid constituents of urine in amounts equivalent to 500 ml. of urine were made up to 10 ml. with redistilled water. Four drops from the 1 ml. pipette gave the ion concentration in a volume of urine equivalent to the amount being tested in the unknowns. (P. 730, Mathews, Physiological Chemistry, 4th ed., Wm. Wood and Co., 1927.)

of the two persistent copper lines. If the difference was 0.5 mm. or less, the line made with the added sodium chloride was used, while any difference greater than 0.5 mm. was read from the line made by the copper alone. Thus using the 'best fit' line the range of recoveries was for urine 92 to 103%, feces 98 to 110%, and food 101 to 107% (table 2). Thompsett ('34), using the carbamate method; obtained recoveries of 85 to 104.6% for urine, 96 to 109.5% for feces, and 98 to 108% for food. McFarlane ('32) who also used the carbamate

TABLE 2

*Recoveries of copper from samples of urine, feces and food*

| SAMPLES DATES | ALONE | | | | WITH 0.005 MG. Cu | | | | RECOVERY | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3889A | 3247.5A | Difference | Concentration | 3889A | 3247.6A | Difference | Concentration | | |
| | mm. | | mm. | mg. | mm. | | mm. | mg. | mg. | % |
| Urine | | | | | | | | | | |
| G.E. 2/4 | 13.0 | 8.4 | 4.6 | 0.0029 | 14.6 | 10.9 | 3.7 | 0.0075 | 0.0046 | 92 |
| F.V. 2/4 | 14.4 | 9.1 | 5.3 | 0.0014 | 14.8 | 10.8 | 3.8 | 0.0065 | 0.0051 | 101 |
| J.E. 2/4 | 12.2 | 8.7 | 3.5 | 0.0012 | 14.4 | 11.7 | 2.7 | 0.0064 | 0.0052 | 103 |
| Feces | | | | | | | | | | |
| G.E. 2/4 | 14.6 | 10.5 | 4.1 | 0.0043 | 14.6 | 11.1 | 3.5 | 0.0092 | 0.0049 | 98 |
| F.V. 2/4 | 14.6 | 10.7 | 3.9 | 0.0055 | 14.6 | 11.2 | 3.4 | 0.0105 | 0.0050 | 100 |
| J.E. 2/4 | 14.9 | 10.9 | 4.0 | 0.0050 | 14.8 | 11.4 | 3.4 | 0.0105 | 0.0055 | 110 |
| Food | | | | | | | | | | |
| G.E. 2/4 | 14.7 | 9.3 | 5.4 | 0.0011 | 14.7 | 10.9 | 3.8 | 0.0065 | 0.0054 | 107 |
| F.V. 2/4 | 14.5 | 9.3 | 5.2 | 0.0015 | 14.5 | 9.8 | 4.7 | 0.0066 | 0.0051 | 102 |
| J.E. 2/4 | 14.7 | 9.4 | 5.3 | 0.0014 | 14.7 | 10.9 | 3.8 | 0.0064 | 0.0051 | 101 |

method, obtained recoveries which were calculated to be from 55 to 120% for organic substances, while Elvehjem and Lindow ('29) using the modified Biazzo method, recovered from 87.9 to 100.6% of copper added to biological materials.

Inasmuch as the same amounts of hydrochloric acid were used in making up both the known and unknown solutions, and since this was always taken from the same supply, no corrections were made for the slight trace of copper indicated by its spectra.

TABLE 3

*Average daily intake, excretion and retention of copper*

| DIET NO. | CASE DATE | FOOD | | COPPER INGESTION | | | | COPPER EXCRETION | | | | RETENTION | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Theoretical [1] | Analyzed | H2O | A.A. | Total | Per kilogram | Feces | Urine | A.A. | Total | Total | Per kilogram |
| | | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| III | G.E. | | | | | | | | | | | | |
| | 6/2 | 1.14 | 0.91 | 0.01 | 0.01 | 0.91 | | 0.41 | 0.09 | 0.00 | 0.50 | 0.41 | |
| | 6/7 | | 0.65 | 0.01 | 0.01 | 0.65 | | 0.39 | 0.08 | 0.00 | 0.47 | 0.18 | |
| | Average | | | | | 0.78 | 0.045 | | | | | 0.303 | 0.017 |
| I | G.E. | | | | | | | | | | | | |
| | 2/4 | 1.19 | 0.75 | 0.01 | 0.00 [2] | 0.76 | | 0.35 | 0.06 | 0.00 [2] | 0.41 | 0.35 | |
| | 2/9 | | 0.83 | 0.01 | 0.00 [2] | 0.84 | | 0.42 | 0.05 | 0.00 [2] | 0.47 | 0.37 | |
| | Average | | | | | 0.79 | 0.047 | | | | | 0.35 | 0.021 |
| III | J.E. | | | | | | | | | | | | |
| | 6/2 | 1.33 | 1.11 | 0.01 | 0.01 | 1.11 | | 0.21 | 0.09 | 0.00 | 0.30 | 0.81 | |
| | 6/7 | | 1.08 | 0.01 | 0.01 | 1.08 | | 0.16 | 0.09 | 0.00 | 0.25 | 0.83 | |
| | Average | | | | | 1.09 | 0.053 | | | | | 0.82 | 0.040 |
| I | J.E. | | | | | | | | | | | | |
| | 2/4 | 1.36 | 1.03 | 0.01 | 0.00 [2] | 1.04 | | 0.51 | 0.03 | 0.00 | 0.54 | 0.50 | |
| | 2/9 | | 1.25 | 0.01 | 0.00 [2] | 1.26 | | 0.57 | 0.02 | 0.00 | 0.59 | 0.65 | |
| | Average | | | | | 1.14 | 0.058 | | | | | 0.58 | 0.030 |
| III | F.V. | | | | | | | | | | | | |
| | 6/2 | 1.30 | 1.39 | 0.01 | 0.01 | 1.39 | | 0.34 | 0.08 | 0.00 | 0.42 | 0.98 | |
| | 6/7 | | 1.14 | 0.01 | 0.01 | 1.14 | | 0.26 | 0.06 | 0.00 | 0.32 | 0.83 | |
| | Average | | | | | 1.27 | 0.061 | | | | | 0.901 | 0.043 |
| I | F.V. | | | | | | | | | | | | |
| | 2/4 | 1.36 | 1.18 | 0.01 | 0.00 [2] | 1.19 | | 0.52 | 0.03 | 0.00 [2] | 0.55 | 0.64 | |
| | 2/9 | | 1.34 | 0.01 | 0.00 [2] | 1.35 | | 0.60 | 0.04 | 0.00 [2] | 0.64 | 0.71 | |
| | Average | | | | | 1.26 | 0.067 | | | | | 0.67 | 0.035 |
| II | G.E. | | | | | | | | | | | | |
| | 2/25 | 1.09 | 1.23 | 0.00 | 0.00 | 1.23 | | 0.53 | 0.06 | 0.00 | 0.59 | 0.64 | |
| | 3/1 | | 1.16 | 0.01 | 0.00 | 1.17 | | 0.50 | 0.05 | 0.00 | 0.55 | 0.62 | |
| | Average | | | | | 1.20 | 0.070 | | | | | 0.63 | 0.037 |
| III | F.V. | | | | | | | | | | | | |
| | 5/12 | 1.30 | 1.51 | 0.01 | 0.01 | 1.51 | | 0.70 | 0.08 | 0.00 | 0.78 | 0.73 | |
| | 5/17 | | 1.24 | 0.01 | 0.01 | 1.53 | 0.076 | | | | | 0.85 | 0.040 |
| | 2/25 | 1.28 | 1.50 | 0.00 | 0.00 | 1.50 | | 0.43 | 0.08 | 0.00 | 0.51 | 0.99 | |
| | 3/1 | | 1.44 | 0.01 | 0.00 | 1.45 | | 0.42 | 0.08 | 0.00 | 0.50 | 0.95 | |
| | Average | | | | | 1.46 | 0.076 | | | | | 0.97 | 0.049 |
| III | J.E. | | | | | | | | | | | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5/17 | | 1.54 | 0.01 | 0.01 | 1.53 | 0.075 | | | | | 0.66 | 0.040 |
| | 2/25 | 1.26 | 1.50 | 0.00 | 0.00 | 1.50 | | 0.43 | 0.08 | 0.00 | 0.51 | 0.99 | |
| | 3/1 | | 1.44 | 0.01 | 0.00 | 1.45 | | 0.42 | 0.08 | 0.00 | 0.50 | 0.95 | |
| | Average | | | | | 1.46 | 0.076 | | | | | 0.97 | 0.049 |
| III | J.E. | | | | | | | | | | | | |
| | 5/12 | 1.34 | 1.58 | 0.01 | 0.01 | 1.58 | | 0.43 | 0.06 | 0.00 | 0.49 | 1.09 | |
| | 5/17 | | 1.69 | 0.01 | 0.01 | 1.69 | | 0.54 | 0.07 | 0.00 | 0.61 | 1.08 | |
| | Average | | | | | 1.63 | 0.079 | | | | | 1.08 | 0.053 |
| II | F.V. | | | | | | | | | | | | |
| | 3/19 | 1.28 | 1.59 | 0.01 | 0.00 | 1.60 | | 0.75 | 0.07 | 0.00 | 0.82 | 0.78 | |
| | 3/22 | | 1.58 | 0.01 | 0.00 | 1.59 | | 0.79 | 0.07 | 0.00 | 0.86 | 0.73 | |
| | Average | | | | | 1.59 | 0.079 | | | | | 0.75 | 0.037 |
| III | J.E. | | | | | | | | | | | | |
| | 4/21 | 1.31 | 1.63 | 0.01 | 0.00 | 1.64 | | 0.84 | 0.06 | 0.00 | 0.90 | 0.74 | |
| | 4/26 | | 1.85 | 0.01 | 0.00 | 1.86 | | 0.68 | 0.06 | 0.00 | 0.74 | 1.12 | |
| | Average | | | | | 1.74 | 0.081 | | | | | 0.92 | 0.046 |
| III | G.E. | | | | | | | | | | | | |
| | 4/21 | 1.11 | 1.44 | 0.01 | 0.00 | 1.45 | | 0.52 | 0.05 | 0.00 | 0.57 | 0.88 | |
| | 4/26 | | 1.43 | 0.01 | 0.00 | 1.44 | | 0.58 | 0.06 | 0.00 | 0.64 | 0.80 | |
| | Average | | | | | 1.44 | 0.083 | | | | | 0.83 | 0.048 |
| II | G.E. | | | | | | | | | | | | |
| | 3/18 | 1.09 | 1.39 | 0.01 | 0.00 | 1.40 | | 0.48 | 0.07 | 0.00 | 0.55 | 0.85 | |
| | 3/22 | | 1.44 | 0.01 | 0.00 | 1.45 | | 0.46 | 0.07 | 0.00 | 0.53 | 0.92 | |
| | Average | | | | | 1.42 | 0.084 | | | | | 0.88 | 0.052 |
| II | J.E. | | | | | | | | | | | | |
| | 3/17 | 1.30 | 1.65 | 0.01 | 0.00 | 1.66 | | 0.41 | 0.08 | 0.00 | 0.49 | 1.15 | |
| | 3/22 | | 1.65 | 0.01 | 0.00 | 1.66 | | 0.45 | 0.07 | 0.00 | 0.52 | 1.14 | |
| | Average | | | | | 1.66 | 0.084 | | | | | 1.14 | 0.058 |
| III | G.E. | | | | | | | | | | | | |
| | 5/12 | 1.14 | 1.45 | 0.01 | 0.01 | 1.45 | | 0.78 | 0.07 | 0.00 | 0.85 | 0.60 | |
| | 5/17 | | 1.50 | 0.01 | 0.01 | 1.50 | | 0.81 | 0.07 | 0.00 | 0.88 | 0.62 | |
| | Average | | | | | 1.47 | 0.085 | | | | | 0.61 | 0.035 |
| II | J.E. | | | | | | | | | | | | |
| | 3/1 | 1.25 | 1.61 | 0.01 | 0.00 | 1.62 | 0.085 | 0.73 | 0.06 | 0.00 | 0.79 | 0.83 | 0.043 |
| III | F.V.[3] | | | | | | | | | | | | |
| | 4/21 | 1.28 | 1.61 | 0.01 | 0.00 | 1.69 | | 0.90 | 0.07 | 0.00 | 0.97 | 0.65 | |
| | 4/26 | | 1.90 | 0.01 | 0.00 | 1.91 | | 0.84 | 0.07 | 0.00 | 0.91 | 1.00 | |
| | Average | | | | | 1.75 | 0.087 | | | | | 0.81 | 0.040 |

[1] Calculated on basis of published tables, Lindow et al. ('29). [2] Estimated value. [3] Slight regurgitation which was not analyzed.

## DISCUSSION OF RESULTS

The copper concentration of all foods was determined from the curve made without the addition of sodium chloride, while most of the urines and feces were read on the line made with sodium chloride. The results are given in table 3. The estimated amounts of copper of the food, based upon the findings of Lindow, Elvehjem and Peterson ('29) are also given. It will be noted that of the eighteen cases studied, six received food which was lower in copper 2 to 33% than the estimated value, while the remaining twelve cases received food which was 10 to 36% higher. Fourteen of these eighteen show differences which fall within the range of experimental error as determined for this study. When these values are compared with the usually accepted 20% range, eleven of the eighteen exceed that amount. Some of the differences between the published and analyzed values may be due to the method employed, since the published copper values for foodstuffs given by Lindow, Elvehjem and Peterson ('29) were determined by the modified Biazzo method with which Thompsett ('34) obtained slightly lower copper values for both food and feces than with the carbamate method. Both McFarlane ('32) and Ansbacher, Remington and Culp ('31) had concluded that the carbamate method give higher values for copper than the Biazzo method. Another factor which may contribute to the difference in the copper content of the diets is the source of the food supply. For example, diet I of the present study, which was thought to be highest in copper because of the use of a whole wheat cereal and breadstuff, was shown on analysis to be the poorest in this element; whereas diets II and III, in which the theoretical values were lower, were found on analysis to have higher values. The substitution of tomatoes for green beans, with the increase in bananas in these two diets, suggests that these foods were responsible for the increased amounts of copper. The variation in copper content of the two food supplies for each double metabolism period usually fell within the range of experimental error. In two instances, this amount was exceeded. In considering the second instance when diet III was

involved, 'new' potatoes were used instead of old ones, and the analyzed copper value was low.

As previously stated, in the only copper balance study with children, Daniels and Wright ('34) employed Elvehjem and Lindow's ('29) modified Biazzo method. The range of copper ingestion in their study was from 1.23 to 2.00 mg., whereas in the present study the copper ingestions ranged from 0.7 to 1.9 mg. per day. On the basis of the child's weight these values represent ingestion levels of from 0.069 to 0.113 mg. in the earlier study and from 0.045 to 0.087 mg. per kilogram of body weight for the present study. In the former study both evaporated and pasteurized milk were used, the higher ingestions being coexistent with the use of evaporated milk which was shown to be relatively high in copper.

With the exception of the last metabolism period the percentage of ingested copper excreted in the urine was quite constant for a given child for all periods, ranging from 5 to 6 in one case (G.E.) and between 2 and 5 in the second (J.E.). These urinary excretions of copper bore no constant relation to the ingestion levels. There is, however, a surprising consistency in the urinary copper excretions in many of the double periods for a given child at a given ingestion level.

The copper content of the feces ranged from 15 to 58% of the ingested copper. These values are considerably lower than the 45 to 85% which was excreted by the subjects used in the study reported by Daniels and Wright ('34).

While the copper retentions varied from one metabolism period to another and were not uniform for a given child, the retentions were usually higher during the period of high copper ingestion. The highest retentions obtained, 53 and 58 mg. per kilogram, were approximately 66% of the copper ingested; whereas Daniels and Wright ('34) found from 15 to 30% of the ingested copper was retained. Possibly the difference in the two experimental procedures may account for the individual responses obtained during a period of 5 months. The present work was concerned with a study of the same three children who had been living in the metabolism

ward during the preceding 4 months and as far as could be determined were well-fed children; whereas the nutritional status of the children of the previous study was unknown. Furthermore, a longer preliminary period preceded each double metabolism period thus allowing sufficient time for better adjustment to changes in the copper content of the diet. Evidence of this adjustment is indicated by the uniformity of the urinary excretions of copper in the two consecutive 5-day periods, as well as by the fact that the fecal copper usually paralleled the changes or fluctuations in the copper content of a given diet.

The maximum retentions, 0.058 and 0.053 mg. per kilogram of body weight, occurred with a diet containing 0.084 and 0.079 mg. of copper per kilogram. These retentions are considerably higher than the figures given by Daniels and Wright ('34) who report retentions of 0.048 and 0.034 mg. per kilogram with ingestion levels of 0.093 and 0.090, respectively. While maximum retentions in the present study were always associated with the higher copper intakes, the retentions were not proportional to the amount of copper ingested. However, the two lowest retentions 0.017 and 0.021 mg. per kilogram were obtained with the two lowest levels of ingestion, namely 0.045 and 0.047. Since higher ingestion levels, 0.085 and 0.087 mg. per kilogram, did not give greater retentions than those possible with an ingestion of 0.053, it is concluded that between 0.053 and 0.085 mg. of copper per kilogram of body weight are required by boys between the ages of 3 and 6 years.

## SUMMARY

Thirty-five copper balance studies have been made with three normal preschool age boys. The spectra of the ash of food, feces, urine, distilled water and acid-alcohol were compared with those produced by solutions of known copper content and the retentions calculated.

The daily urinary copper excretion was fairly constant for a given child, averaging 4% of the ingested copper.

Fifteen to 58% of the ingested copper was excreted by the way of the alimentary tract.

The lowest copper retentions occurred with the lowest copper intakes, while the highest retention, 0.058 mg., was obtained with an ingestion which was close to the maximum level of copper consumed, namely 0.084 mg. per kilogram of body weight. Since higher retentions did not occur with the higher ingestions tested, it is concluded that between 0.053 and 0.085 mg. of copper per kilogram of weight are required by boys between the ages of 3 and 6 years.

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# THE HISTOPATHOLOGY OF NEUROMALACIA AND 'CURLED TOE' PARALYSIS IN THE CHICK FED LOW RIBOFLAVIN DIETS [1, 2, 3]

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EIGHT FIGURES

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In a chick assay of certain strains of yeasts for their riboflavin content a number of animals developed 'curled toe' paralysis At post mortem these chicks showed bilateral hypertrophy of the sciatic nerve. Histologic examination revealed degenerative changes in these nerves. It seemed that the pathology was associated with the diet and that a low riboflavin intake was responsible for the condition observed in these nerves. In order to pursue this suggestion further a series of experiments was made.

The riboflavin low ration of Bethke, Record and Wilder ('37) was used as the basal ration throughout these experiments. It was composed of:

| | | | |
|---|---|---|---|
| Yellow corn | 59 | NaCl | 1 |
| EtOH extracted casein | 12 | $Ca_3(PO_4)_2$ | 1 |
| Wheat bran | 5 | $CaCO_3$ | 1 |
| Whole wheat | 20 | Cod liver oil (Abbott's)[4] | 1 |

Routine histologic observations were made on the sciatic and inferior branch of the brachial plexus, the brachial and

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[2] These studies were aided in part by a grant from the Wisconsin Alumni Research Foundation.

[3] A preliminary report of a portion of these studies was made in Poultry Science, July, 1938.

[4] Abbott's cod liver oil (U.S.P. XI), medicinal grade.

cervical segments of the spinal cord, cerebellum, portions of the brain stem, cerebrum, kidney, liver and occasionally the thyroid, thymus, suprarenal glands, and leg muscles. The myelin sheaths of the peripheral nerves were studied by means of plane polarized light between crossed Nicols as well as the usual technics of Marchi and Sudan III. Axis cylinders, neurofibrillae and muscle nerve endings were studied by means of the Bodian ('36) silver impregnation method. Nissl preparations were made with gallocyanin according to Einarson ('32). All other tissues were stained with H and E.

In the first experiment two lots of animals each containing seven 1-day-old White Leghorn chicks were used. Lot 1 was given the basal ration only and lot 2 was given dried oat grass no. 208.[5] Three chicks of lot 2 were fed 2% of this grass supplement, two were given 4%, and two were given 6%. The chicks on the basal ration alone all developed 'curled toe' paralysis, four with severe symptoms and three with mild symptoms. Those with severe symptoms gave evidence of neuromalacia upon post mortem and histologic examination. The two chicks receiving 2% oat grass developed severe and earlier symptoms than the lot 1 chicks. The 4% level of oat grass did not protect because symptoms of 'curled toe' developed. Microscopic examination confirmed the gross symptoms. The two chicks on the 6% level were apparently protected as far as gross symptoms were concerned but microscopic examination showed that the sciatic nerve was damaged in one of these birds. This experiment seemed to indicate definitely that a marked neuropathology accompanied 'curled toe' paralysis, or neuromalacia. However, the carrier of riboflavin which we had chosen was not fed at a high enough level to give complete protection against riboflavin deficiency. Since grass carries many nutritional factors, this experiment did not specifically isolate riboflavin as the protective agent.

[5] Obtained from the American Butter Company of Kansas City.

A second experiment was then made with day-old chicks divided into two lots of ten birds each. Lot 1 received the basal ration only and lot 2 received the basal ration plus 300 micrograms of crystalline riboflavin (Hoffman-La Roche) per 100 gm. of ration. The chicks on the basal ration developed various degrees of neuromalacia by the fifth to the seventh weeks. The gross symptoms of the disease were similar to those described by Norris et al. ('30) and Bethke et al. ('31). The severe cases showed typical hypertrophy in the sciatic and brachial nerve trunks. Two characteristic forms of the syndrome were observed. A rapidly acute form developed with marked dystonia and paralysis of the legs. The typical 'curled toe' condition was observed infrequently in this form. A more slowly developing disorder occurred in which the toes gradually flexed inward as paralysis developed. In both types ataxia developed followed by dystonia, paresis and finally complete paralysis of the foot, or leg, or both. Such paralysis usually developed bilaterally although it was not uncommon to find only one branch of the sciatic nerve trunk affected. In acute and severe cases the affected nerves frequently reached a diameter several times their normal size. This condition was readily discernible grossly by the size and the characteristic brownish-yellow translucency of the affected nerves.

The chicks in lot 2 which received added riboflavin were remarkably well protected against either gross symptoms or microscopic lesions. Two chicks died early in the experiment and were not studied. Of the remaining eight, five were used for histologic purposes. No manifestations of paralysis or 'curled toe' were observed in this lot during the first 7 weeks of life. Growth was somewhat slow but the average weight of the chicks attaining 7 weeks of age was approximately 350 gm. The sciatic nerve and brachial nerves were entirely free from any microscopic evidence of neuromalacia. It was somewhat disconcerting, however, to find a scattered positive Marchi reaction in the spinal cord of three of these chicks.

A similar experiment was repeated with six birds per lot. Lot 1 was given the basal ration plus 300 micrograms of riboflavin per 100 gm. of ration, and lot 2 was given the basal ration only. One of the chicks died in lot 1. One of the remaining control chicks developed a slight curling of the central digits of the foot and a second developed dermatitis. The latter was discarded since it was obviously affected by more than a riboflavin deficiency. This indicated that our basal ration was at least borderline in the chick antidermatitis factor. Slight evidence of histologic damage was noted in the nerve and cord from the animal with the curled central digit. This would seem to indicate that the level of riboflavin used in the experiment was either inadequate, or that the storage was low in the chick at hatching. The chicks in lot 2 on the basal ration alone showed evidence of 'curled toes' in all cases. Four of these were severe and two were mild. Five of these chicks also showed positive Marchi reactions in the spinal cord.

Since the previous experiment had shown that dermatitis developed in the chicks on the basal ration with added riboflavin, a fourth experiment was planned. Three lots of five chicks each were used. Lot 1 received the basal ration only, lot 2 received the basal ration plus a chick antidermatitis factor concentrate equivalent to 4% liver, the third lot was given the basal ration plus 300 micrograms of riboflavin per 100 gm. of ration. Only three chicks in lot 1 survived to develop a riboflavin deficiency. The chicks in lots 1 and 2 without added riboflavin developed neuromalacia. Those in lot 1 (basal only) all had curled toes while those in lot 2 with the added chick antidermatitis factor were without curled toes, yet both lots exhibited equally severe paralysis. Histologic examination showed marked degeneration in the myelin sheaths of the sciatic nerves and in many cases the axis cylinders as well. Lot 3 which received the added riboflavin was completely protected from either gross or microscopic evidence of neuromalacia. Again it was observed that a scattered positive Marchi was evident in the spinal cords

of some birds in all lots on this experiment irrespective of their dietary regimen. It appeared that the addition of the antidermatitis factor concentrate definitely reduced the amount and degree of degeneration in the spinal cords of chicks in lot 2. No dermatitis was observed in this experiment.

A fifth experiment was then set up to test again the results obtained in experiment 4 and also to observe the effect of increasing the level of riboflavin. The experimental plan with six birds per lot was as follows: lot 1 basal ration only, lot 2 basal ration plus 400 micrograms of riboflavin per 100 gm. of ration, lot 3 basal ration plus a chick antidermatitis factor concentrate equivalent to 4% liver, lot 4 basal ration plus riboflavin as in lot 2 plus the antidermatitis factor as in lot 3. The addition of the antidermatitis factor concentrate was discontinued after the first 5 weeks of the experiment, and the birds allowed to continue on the basal only until they were removed from the experiment during the sixth week. At the beginning of the sixth week no 'curled toe' was observed in any of the chicks. Two chicks had died in lot 2 and a fourth was under weight and unthrifty. There was no uniformity of growth in any of the lots, apparently attesting to the wide variation in this hatch of chicks. The chicks in lot 4 were superior in appearance with the exception of one light weight chick. No outward symptoms of 'curled toe' paralysis were detected in any of the lots.

Three of the chicks in lot 3 were stricken with acute intermittent leg paralysis during the last week on the experiment. The birds were disinclined to walk except when forced. They assumed a squat position when at rest and walked in this position at times by the aid of their wings. In these animals the wings were inclined to droop as though they could not be held in the proper position. Drooped wings were observed in lot 1 but not in the lots receiving riboflavin. At this point it seemed that this experiment was abortive in its original plan of producing neuromalacia and that either the riboflavin in the basal ration was sufficient to carry these

chicks, or that some conditioning factor had influenced the requirements of the chicks in this particular hatch. The chicks were accordingly removed from the experiment at 6½ weeks of age and histologic data obtained. Microscopic examination of these chicks showed that the experiment was not as disappointing as we first believed. Lot 1 which received the riboflavin low diet produced five cases that showed mild, or early, myelin degeneration of the sciatic nerve and two cases of spinal cord degeneration. Lot 2 on the other hand received added riboflavin and the four chicks examined showed normal nerves and spinal cords. Lot 3 which received the added antidermatitis factor concentrate all showed very positive sciatic nerve degeneration although only one chick in the lot was definitely paralyzed in the legs at the termination of the experiment. Only one case showed mild spinal cord degeneration. This would seem to demonstrate again that the antidermatitis factor concentrate prevented or greatly reduced the myelin degeneration in certain areas of the spinal cord but it was not effective in reducing the changes in the sciatic nerve. This cannot be regarded as a crucial experiment in this respect since severe neuromalacia was not produced in this hatch of chicks. Lot 4 chicks which received both the riboflavin and the antidermatitis factor concentrate were entirely free from any neuropathology of either peripheral nerves or spinal cord.

In order to subject the observation that the chick antidermatitis factor was involved in some manner with the degeneration of the spinal cord mentioned above, an experiment was designed to test this point further. Accordingly the following experiment was made using basal ration no. 241H used by Mickelsen, Waisman and Elvehjem ('38) as a test ration low in the chick antidermatitis factor.

Lot 1 Basal ration only.
Lot 2 Basal ration + nicotinic acid + riboflavin.
Lot 3 Basal ration + riboflavin
Lot 4 Basal ration + chick antidermatitis factor.
Lot 5 Basal ration + antidermatitis factor + riboflavin.
Lot 6 Basal ration + 15% artificially dried grass.

Seven chicks examined from lot 1 receiving the basal ration only showed considerable degeneration in the spinal cord and four cases indicated mild degeneration of the sciatic nerve. Lot 2 (five chicks examined) showed cord degeneration but no changes in the sciatic nerve. The same was found where riboflavin was fed alone, thus indicating that the sciatic nerve degeneration noted in the first lot was due to a riboflavin deficiency. It appears that ration 241H may be marginal in its riboflavin content. If the antidermatitis factor was supplied, only very mild cord changes, if any, were present, but occasionally the sciatic nerve was degenerated. General observations led to the interpretation that the antidermatitis factor stimulated appetite in response to growth and thereby raised the riboflavin ingestion sufficiently to avoid much of the degenerative changes found in the sciatic. Where both riboflavin and the antidermatitis factor was fed no degeneration was observed in either cord or nerves. Fifteen per cent dried grass protected the sciatic nerve, but it was inadequate to protect completely against the cord pathology.

This experiment was repeated later with three groups of six chicks each. One group was given the basal ration only, one the basal ration plus riboflavin, and the third the basal ration plus the chick antidermatitis factor. Without riboflavin additions some degenerating fibers were found in the sciatic nerve. The addition of riboflavin prevented the degenerative changes but did not prevent the cord pathology in more than 75% of the cases. The addition of the antidermatitis factor again largely reduced the appearance of changes observed in the spinal cord, but it was ineffective in preventing occasional changes in the sciatic. These experiments are an early indication of what may be expected but a more detailed investigation must be made to clarify them satisfactorily.

From the foregoing series of experiments it is seen that riboflavin appears to have a specific action in preventing nerve degeneration in the growing chick. The histopathologic changes observed in this disease are herewith described.

The histologic changes observed were confined primarily to the nervous system. The sciatic nerve trunk was affected in 95% of the cases indicating a higher incidence than that demonstrated by gross symptoms. The nerve was hypertrophied and exhibited myelin degeneration in one or more of its branches. The nerve fibers were widely separated, apparently by the accumulation of metabolic products within the myelin sheath. Such fibers were unmistakably degenerating as indicated by the decrease in birefringent material demonstrable between crossed Nicols in the polarizing microscope (figs. 1 and 2). Portions of the same nerve trunk treated with osmic acid according to the rapid Marchi method showed a marked positive reaction indicative of degenerative changes. In our hands these two methods were in very close agreement on all the nerves studied in this series of experiments (figs. 3 and 4). Silver preparations showed axis cylinder swelling, fragmentation and Schwann cell proliferation (figs. 5 and 6). Similar changes were present in many of the brachial nerve trunks which were examined.

The addition of riboflavin to the basal ration was effective in preventing neuromalacia and produced practically 100% normal histology in the peripheral nerve trunks. In only one

Fig. 1 Chick no. 579, fed the basal diet + riboflavin. Normal distribution of birefringent material. Crossed Nicols and polarizing microscope. × 440.

Fig. 2 Chick no. 583, fed the basal diet only. Degeneration of myelin as shown by the disturbed distribution of birefringent material in the medullary sheath. Crossed Nicols and polarizing microscope. × 440.

Fig. 3 Chick no. 579, fed a low riboflavin diet + 100 micrograms of crystalline riboflavin per 100 gm. of ration. Normal sciatic nerve. Marchi. × 660.

Fig. 4 Chick no. 583, fed low riboflavin basal diet only. Notice myelin degeneration in the fibers of the sciatic. Marchi. × 660.

Fig. 5 Chick no. 625, fed the basal diet + riboflavin. Sciatic nerve, silver impregnation of axis cylinders. × 440.

Fig. 6 Chick no. 583, fed the basal diet only. Sciatic nerve showing axis cylinder fragmentation and Schwann cell proliferation. Silver. × 660.

Fig. 7 Chick no. 626, fed the basal diet only. Showing evidence of gliosis and chromatolysis in the brachial segment of the spinal cord. Gallocyanin. × 440.

Fig. 8 Chick no. 630, fed the basal ration only. Muscle and muscle end plate degeneration. Silver. × 660.

Figures 1 to 8

case in these experiments where riboflavin was fed was there unmistakable evidence of myelin degeneration in the sciatic nerve.

The neuropathology of the cord was marked by myelin reactions with osmic acid varying from a few scattered fibers to rather numerous positive fibers. These observations were made upon longitudinal sections. Gross sections and observations on the longitudinally cut sections showed that the degeneration was not restricted to specific fiber tracts but seemed to be confined to and scattered throughout the posterior and posterolateral regions of the cord. Silver and Nissl preparations indicated various degrees of axis cylinder pathology, fibrillae fragmentation, nuclear changes, Nissl chromatolysis and gliosis (fig. 7). No particular structure of the cord seemed to show any regularity in susceptibility to the deficiency as was the case in the peripheral nerves where the myelin sheath was practically always affected.

Several muscles of the leg, among them the gastrocnemius, the gluteal group and the vastus internus were impregnated with silver for nerve endings and muscle fiber histology. The nerve end plates in many of these cases were swollen, bulbous and appeared to be in the process of degeneration (fig. 8). No gross muscle dystrophy was apparent although not infrequently single muscle fibers were completely degenerated (fig. 8).

The liver was normal in size and color but generally the gall bladder was distended. Occasionally congestion was plainly evident from the appearance of the blood vessels. The 'yellow liver' associated by Sebrell et al. ('38) with riboflavin deficiency in dogs was not observed in these experiments except in rare, isolated cases. Such yellow livers have invariably been associated with chicks fed diets low in vitamin $B_4$ (Engel and Phillips, '37). A few such livers did occur and there was no explanation for them.

The thymus was found to be in various stages of involution in these chicks. Those lobes which were taken for study were usually showing congestion and premature atrophy.

Microscopic examination confirmed these observations. While it is recognized that the thymus gland in the chick is a variable organ it seems that riboflavin low diets tend to cause developmental failure, early involution, or atrophy of this gland. The kidney, thyroid, suprarenal gland and the various sections of the brain and brain stem which were examined showed no alteration by the technic employed.

## DISCUSSION

The riboflavin used in these experiments was the synthetic product made by Hoffman-La Roche, Inc. It appears from these data that either the riboflavin requirements of the growing chick with this source of riboflavin is greater than 300 γ per 100 gm. of ration in the first 3 weeks of life or that destruction of the riboflavin occurred after it was added to the ration. These data do not preclude the possibility that the action of the riboflavin was indirect, but the bulk of evidence supports the view that neuromalacia in the chick is caused from a riboflavin deficiency and that it can be prevented by the addition of adequate riboflavin to the diet.

The histopathology observed in these experiments clarifies somewhat the apparent discrepancies observed by several workers engaged in studying riboflavin requirements. Not infrequently investigators have failed to obtain paralysis on riboflavin low diets and often paralysis has developed and then for no apparent reason whatever recovery took place without the addition of riboflavin supplements. From these microscopic studies it appears that the incidence of anatomical change is higher than the gross symptoms would indicate. Further, it seems reasonable to suggest that those cases which exhibited only mild myelin degeneration could recover without permanent injury, while those cases which showed both myelin and axis cylinder degeneration would remain permanently disabled regardless of dietary therapy. It is estimated from these data that approximately 50% of these cases would not fully recover and that 40% might recover fully and the remaining 10% might or might not recover. Thus from histopathologic studies one might expect

recovery from riboflavin deficiency in the chicks in about 50% of the cases.

## SUMMARY AND CONCLUSIONS

It has been demonstrated that a low riboflavin diet may cause two types of a nutritional deficiency. One of these produces a rapidly acute paralysis characterized as neuromalacia and a more slowly developing form which causes 'curled toe' paralysis in the growing chick. These symptoms can be prevented by the addition of crystalline riboflavin to the basal diet.

This nutritional deficiency disease is accompanied by specific neuropathology of the main peripheral nerve trunks. In the acute form there is hypertrophy and change in appearance which can be readily observed by gross inspection. Degenerative changes appear in the myelin of the nerves, accompanied by Schwann cell proliferation and axis cylinder swelling and fragmentation. Histologic changes also occur in the spinal cord where myelin changes, gliosis, and chromatolysis have been observed. Neuro-muscular end plate degeneration and muscle fiber degeneration frequently accompany the paralysis.

Histologic examination of the sciatic nerve from chicks fed artificially dried young grass shows that a 6% level of oat grass was marginal in its flavin content for the growing chick. It was found that addition of the antidermatitis factor to the low flavin basal diet considerably reduced the histopathology of the spinal cord. This suggests that the low riboflavin diet used in this series of experiments may also be low or marginal in the antidermatitis factor. Chicks fed ration 241H also showed improvement in the histopathology of the spinal cord when the antidermatitis factor was fed but the sciatic nerve was not protected. The latter was protected by the addition of crystalline riboflavin. Histologic examination also indicates that the addition of 300 micrograms of riboflavin to each 100 gm. of the basal diet was inadequate for complete protection of the chick during the early weeks of life.

It is concluded from these studies that riboflavin is necessary in the growing chick for the normal function and maintenance of the nervous system, particularly the main peripheral nerve trunks and it may be specifically associated with myelin metabolism.

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# TOXICITY OF ASPERGILLUS SYDOWI AND ITS CORRECTION [1]

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FIVE FIGURES

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In a previous publication (Gorcica, Peterson and Steenbock, '35 a) it was shown that rats did not thrive on a diet in which all proteins and B vitamins were supplied by the mycelium of Aspergillus sydowi. It had been shown earlier (Gorcica, Peterson and Steenbock, '35 b) that the same mold contained all the known members of the vitamin B complex. Since it was demonstrated (Gorcica, Peterson and Steenbock, '35 a) that various crude protein supplements improved the ration, it was tentatively concluded that some indispensable amino acid was lacking in the mycelium. Further work has shown that this theory is no longer tenable, and that the problem is much more complex than was at first anticipated. Certain experiments that point to the presence of a toxic substance in the mold will be described in this paper. In addition, various organic and inorganic supplements which render the mycelium innocuous by adsorption of the toxic material or by some unknown mechanism will be discussed.

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## EXPERIMENTAL

### *Basal diet and feeding technic*

The basal diet consisted of dried, ground mycelium of Aspergillus sydowi, 50; dextrin, 25; butterfat, 10; sucrose, 10; cod liver oil, 1; Wesson salts, 4. The technic of growing and harvesting the mold was the same as previously described ('35 a). After harvesting, the mycelium was dried at 60°, ground and stored for use. The various supplements which were fed were incorporated in the basal diet in place of some of the dextrin. The rations were supplied to the animals ad libitum. Young male albino rats from our stock colony were taken when they weighed approximately 50 gm. and started on experiment. Each group was made up of three or more animals. These were weighed at weekly intervals. Because of variations in the animals or the mycelium, or both, it was found essential to perform the experiments at least twice before results could be accepted with certainty.

A typical response to the basal diet is shown in figure 1. At times growth was not so good as this, but an occasional group of animals showed a better response. Deaths usually began to occur about the sixth week. Prior to death the animals showed no symptoms of disease. Autopsy of the dead animals revealed no apparent abnormalities.

### *Protein and amino acid supplements*

Of all the proteins tested, casein proved to be the best. A 10% supplement of this protein resulted in very good growth. On the other hand, 10% supplements of lactalbumin, edestin or heated egg white did not prove so effective. With these proteins, growth was good for the first few weeks but soon ceased. In some cases death occurred. The response to lactalbumin, which was typical of the other proteins, is shown in figure 1. The response to casein is also shown. Growth data for all the proteins are contained in table 1.

Various amino acids, singly and as mixtures, were tested for their ability to produce growth. Additions of leucine,

methionine, cystine and a mixture of all three did not give significantly better growth than did the basal alone. Similar results were obtained with supplements of casein hydrolysates and fractions thereof. However, survival time was increased when nutritionally essential amino acids were added to the ration (fig. 1 and table 1).

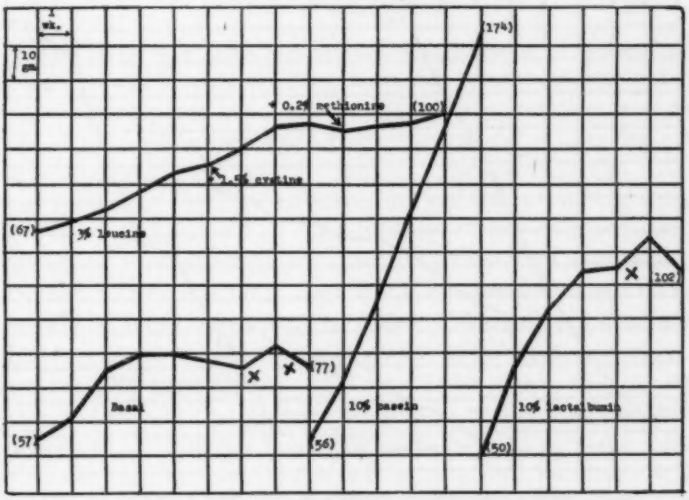


Fig. 1 Growth curves of animals on protein and amino acid supplements.

TABLE 1

*Value of proteins and amino acids as supplements*

| SUPPLEMENT | LEVEL | AVERAGE INITIAL WEIGHT | AVERAGE GAIN IN 6 WEEKS |
|---|---|---|---|
| | % | *gm.* | *gm.* |
| Casein | 10 | 56 | 129 |
| | 5 | 57 | 70 |
| Lactalbumin | 10 | 50 | 52 |
| Edestin | 10 | 50 | 44 |
| Egg white | 10 | 54 | 51 |
| dl-leucine (synthetic) | 3 | 68 | 24 |
| dl-leucine + cystine | 3 L, 0.65 C | 68 | 36 |
| Leucine + cystine + methionine | 3 L, 1.5 C, 0.2 M | 95 | 10 (4 weeks) |

By far the most potent supplement found was whole, air-dried hog liver (fig. 2). Two per cent of this material always gave very good growth. In no case did any animals die on the ration with this supplement. Even an addition of 0.5% liver gave definitely better growth than was obtained on the basal ration alone or with amino acid supplements (table 2).

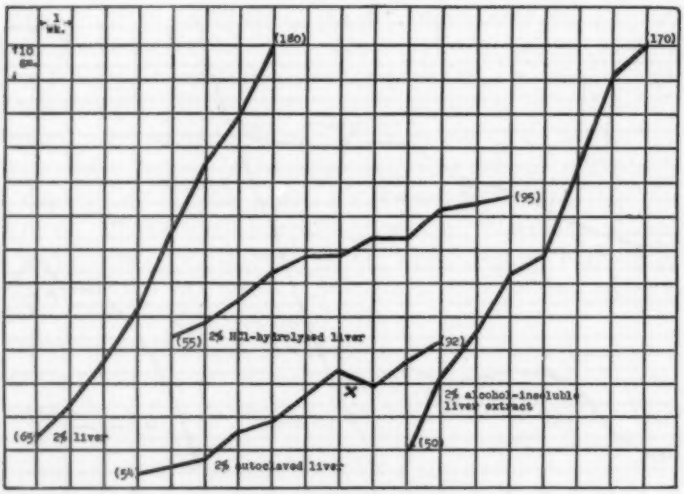


Fig. 2 Growth responses of animals to liver and fractions of liver.

TABLE 2

*Properties of the protective factor of liver*

| FRACTION | LEVEL | INITIAL WEIGHT | GAIN IN 6 WEEKS | DEATHS IN 10 WEEKS |
|---|---|---|---|---|
| | % | gm. | gm. | |
| Whole liver | 2 | 65 | 96 | 0 |
| | 0.5 | 62 | 48 | 0 |
| Autoclaved liver | 2 | 54 | 28 | 1 |
| HCl hydrolyzed liver | 2 | 54 | 30 | 0 |
| 80% EtOH extract of dry liver | 12 | 53 | 40 | 4 [1] |
| Residue after EtOH extraction | 6 | 59 | 75 | 0 |
| $H_2O$ extract of fresh liver | 2 | 76 | 80 | 0 |
| Residue after water extraction of fresh liver | 2 | 84 | 102 | 0 |
| Residue after 2% NaOH extraction of dry liver | 2 | 46 | 118 | 0 |

[1] Group contained four rats.

Indeed, the first indication that the liver owed its potency to more than its content of known amino acids was the discovery that hydrolysis with 25% HCl destroyed its growth-promoting activity. This loss of activity was not due to destruction of tryptophane, for the mold has been shown to contain this amino acid (Woolley and Peterson, '37).

### *Liver fractionations*

Since liver was the most potent supplement, it seemed advisable to use it as a starting material for the concentration of the active principle. Before actual fractionation was begun, some experiments were performed to determine the stability and solubility of the factor. Autoclaving the dried liver for 8 hours at 120° destroyed its growth-promoting activity (fig. 2 and table 2). The lability of the factor to acid hydrolysis was noted above. Ethyl alcohol did not remove the factor from dry liver. On the other hand, hot water extraction of fresh liver removed some of the activity, but most of it remained in the residue. Even alkali removed only a part of the potency from dried liver; the alkali residue was quite active. At best it was possible to remove only a small fraction of the potency from liver with various solvents. The extracted residue was always nearly as potent as whole liver.

A more readily available source of the growth-promoting factor was found in the alcohol-insoluble portion of an aqueous extract of liver.[2] This liver fraction is a by-product in the commercial preparation of concentrates used for the treatment of pernicious anemia. This material was active at a 2% level.

*a. Dialysis.* When the above alcohol-insoluble liver fraction was suspended in water and dialyzed against distilled water, the activity remained, to a considerable extent, in the non-dialyzable portion, while the dialysate was relatively inactive (fig. 3).

[2] We wish to thank Dr. David Klein of the Wilson Laboratories for gifts of this material.

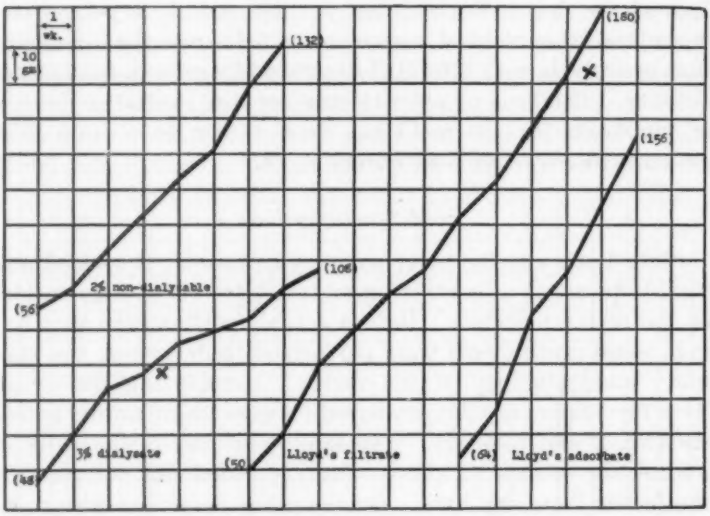


Fig. 3 Growth curves of animals receiving liver fraction supplements.

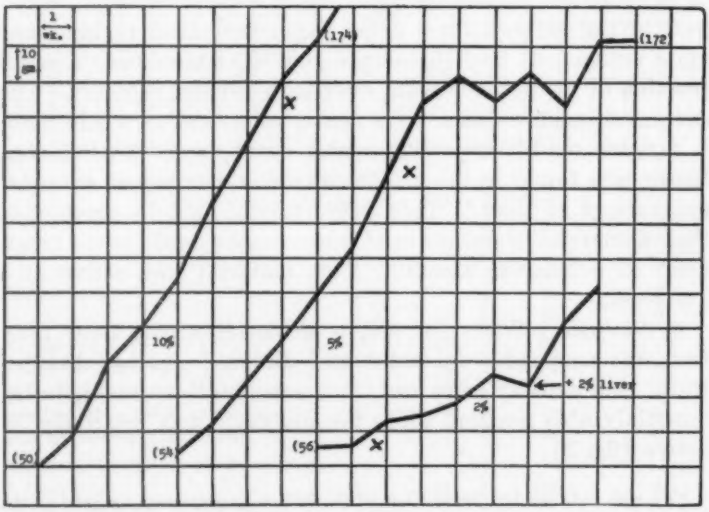


Fig. 4 Growth responses to various levels of Lloyd's reagent.

*b. Adsorption.* Various unsuccessful attempts to adsorb the active principle on a variety of protein adsorbents (aluminum hydroxide, norit and Lloyd's reagent) were made. Since no purification was achieved by these procedures they will not be described. However, it was the use of one of these, Lloyd's reagent, which led to a significant finding regarding the nutritive shortcomings of the mycelium. When aqueous extracts of liver were adsorbed with Lloyd's reagent, the adsorbate as well as the filtrate was quite active (fig. 3). Pyridine eluates from the Lloyd's reagent adsorbates were inactive. It was then found that addition of Lloyd's reagent itself to the basal

TABLE 3

*Supplementing activity of various inorganic adsorbants*

| ADSORBANT | LEVEL | INITIAL WEIGHT | GAIN IN 4 WEEKS | DEATHS |
|---|---|---|---|---|
| | % | gm. | gm. | |
| Lloyd's reagent | 10 | 51 | 54 | 1 |
| Norit | 10 | 48 | 11 | 0 |
| Calcium carbonate | 5 | 48 | 16[1] | 0 |
| Ferric hydroxide | 10 | 50 | 17[1] | 0 |
| Aluminum hydroxide | 10 | 58 | 12 | 0 |
| Permutit | 10 | 54 | 20 | 0 |
| Silica gel | 10 | 46 | 20[1] | 0 |
| Fuller's earth | 10 | 45 | 20 | 2 |

[1] Gain in 3 weeks.

diet gave very good growth. Ten per cent of this material proved about as effective as 10% casein or 2% liver (fig. 4 and table 3).

*Growth-promoting effect of various adsorbents*

Several inorganic adsorbents were next fed in an attempt to determine the specificity of Lloyd's reagent in producing good growth responses. Of all the adsorbents tested, only Lloyd's reagent proved effective. Ten per cent norit, 5% calcium carbonate, 10% ferric hydroxide, 10% silica gel, 10% permutit, 10% aluminum hydroxide, or 10% fuller's earth (Cenco) had no beneficial effect (table 3). Ten per cent

Lloyd's reagent gave very good growth, 5% was not so effective, and 2% was ineffective (fig. 4). Since ignition of the reagent did not decrease its effectiveness, it is impossible to ascribe its activity to contamination with some organic material. In some cases, even though the animals were growing at a rapid rate on the basal supplemented with Lloyd's reagent, sudden death would intervene. No abnormalities were observed either before or after death. Animals which had attained a weight of even 180 gm. would occasionally be thus stricken. This disturbing feature has frustrated our attempts at further purification of the active principle of liver.

The success with Lloyd's reagent led us to try the effect of this supplement in other chronically-toxic diets. However, 10% of the reagent in lathyrism-producing rations (Geiger, Steenbock and Parsons, '33) in mushroom rations (Quackenbush, Peterson and Steenbock, '35) and in raw egg white rations [3] (Parsons and Kelly, '33) had no beneficial effects.

*Fractionation of the mycelium*

The effectiveness of Lloyd's reagent in producing growth suggested that the mycelium contained some poisonous substance which was tenaciously adsorbed by the reagent before it could be absorbed from the tract. If this were true, it should be possible to remove the toxic substance from the mycelium, and thus produce a 'detoxified' ration which would give good growth. This was done in the following manner. Fresh mycelium was lifted from the medium, immediately ground, and dropped into boiling water. The mixture was heated until the temperature rose to 90° in order to destroy proteolytic enzymes. It was then cooled and filtered. The filtrate was stirred with Lloyd's reagent (275 gm. per kilogram of dry mold substance taken), and after several hours the adsorbate was removed in the centrifuge. The filtrate was concentrated under reduced pressure to a sirup, poured on the water-extracted residue, and the mixture was dried before

[3] We wish to thank Dr. Helen T. Parsons for conducting the experiments with raw egg white rations.

a fan. This dried material was then substituted for the fifty parts of mycelium in the basal ration. In some cases, excellent growth was obtained with this 'detoxified' ration; in others, growth was only fair (fig. 5). Since the Lloyd's reagent would remove thiamin and riboflavin from the mold extract, it seemed likely that the poor growth obtained in some cases was due to a lack of some of the vitamin B complex and that the excellent growth obtained at other times was due to

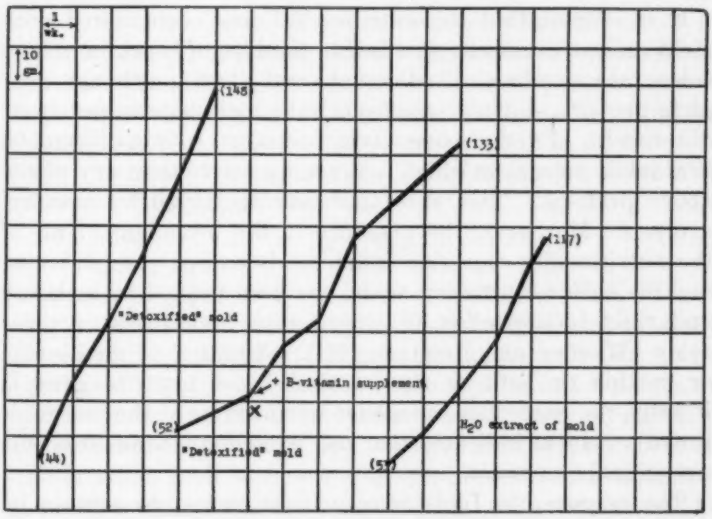


Fig. 5 Growth responses to mold fractions.

the fortuitous incomplete extraction of these vitamins. Supplementation of the 'detoxified' ration with a source of B vitamins (2% of the 92% alcohol-soluble portion of liver extract) gave good growth. This alcoholic extract failed to supplement the basal mold ration, and hence carried none of the protective factor found in whole liver.

Attempts to elute the toxic substance from the Lloyd's reagent adsorbate with barium hydroxide or with pyridine did not prove successful.

Since it was possible to extract the toxic substance from the mycelium with water, attempts to increase the toxicity of the ration seemed advisable. However, when the water extract of the mycelium was added to the basal (extract of 50 gm. of mold added to 100 gm. of ration), the animals always grew better than on the basal alone (fig. 5). A possible explanation of this surprising result will be discussed below.

## DISCUSSION

It is evident that the nutritive failures encountered with mold rations cannot be ascribed to the lack of essential amino acids, for egg elbumin, lactalbumin and edestin, although complete proteins, did not supplement the mold as adequately as did casein. Furthermore, liver contains a non-dialyzable, heat-labile substance which is far more active than any of the above proteins. This substance was destroyed by acid hydrolysis. Moreover, the presence in the mycelium of all of the nutritionally essential amino acids except phenylalanine and the sulfur-containing acids, has been proved by isolation and rigid identification of considerable quantities of these acids (Woolley and Peterson, '37). Addition of methionine or cystine or both to the basal did not improve growth. Finally, the remarkable response to additions of the inorganic Lloyd's reagent indicated that the mycelium contained all the nutritional essentials.

The response to Lloyd's reagent is easiest to explain by postulating a toxic substance in the mycelium. The action of the reagent then seems to be the adsorption of the harmful material before it can be absorbed from the tract. The experiments on 'detoxification' of the mycelium with Lloyd's reagent in vitro support these views.

It thus appears that there are two dietary means of combating the harmful effects of the mycelium. One of these is the addition to the ration of an unknown organic substance (or substances) present in certain proteins, but much more abundant in whole liver. The mode of action of this factor is entirely unknown. The other involves the adsorption of

the harmful mycelial constituent before it can be adsorbed in the tract. This is accomplished by feeding a specific, inorganic substance, Lloyd's reagent.

Certain points remain obscure. Why did the addition of an aqueous extract of the mycelium, which apparently contained the toxic substance, improve the ration? And why did the addition of certain amino acids and protein hydrolysates give some improvement, especially in extending the time of survival? While the answers to these questions are not known, a probable explanation is the following. The mold in the basal ration supplied a suboptimal amount of amino acids. On such a marginal protein intake, the animals succumbed more easily to the action of the toxic principle. When better nitrogen nutrition was provided by the addition of mold extract, or mixtures of amino acids, the animals were better able to combat the toxic substance.

## SUMMARY

The nutritive failure of rats fed on a ration in which all protein and B vitamins were supplied by the mycelium of Aspergillus sydowi is not the result of an amino acid deficiency. Addition of liver to the basal was the most effective means of rendering the ration suitable for growth; casein was less effective, and lactalbumin, egg white, edestin and mixtures of certain amino acids were only slightly effective. Fractionation of a water extract of liver indicated that the potency resided in a high molecular weight substance. The factor was destroyed by autoclaving or by acid hydrolysis. Vitamin B supplements were ineffective.

The ration could also be made suitable for growth by the addition of Lloyd's reagent. Other adsorbents were not effective. These results indicated that there was a toxic substance in the mold. Further proof of this theory was that it was possible to obtain growth on the mold ration by adsorbing a water extract of the mycelium with Lloyd's reagent.

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# THE EFFECT OF EXERCISE AND CHILLS ON HEAT LOSS FROM THE NUDE BODY [1]

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THREE FIGURES

(Received for publication June 16, 1938)

Heat production in the human body during periods of exercise has been studied extensively. Atwater and Benedict ('05) in Middletown, Benedict and his associates ('28) of the Nutrition Laboratory in Boston, Leonard Hill ('19) and his associates in England, and many others have made valuable contributions. Chills have been studied by Cannon, Querido, Britton and Bright ('26) and Swift ('32). DuBois ('37) has added more details in the analysis of results obtained on a normal man who imitated a malarial chill. The main contribution of this present study is due to the fact that it is only by combining a respiration calorimeter with a radiometer that it becomes possible to measure surface temperature changes, separate radiation from convection and follow the balance between heat production and heat loss.

The experimental method has been described (Hardy and DuBois, '38). Two normal men served as subjects and were exposed nude under basal conditions in the calorimeter at temperatures from 22.8°C. to 30°C. The experimental period was usually divided as follows: a preliminary period, 40 to 60 minutes, quiet in calorimeter; a first hour basal; an exercise or chill period; and a last hour recovery period. The

[1] Clinical Calorimetry No. 52.

basal periods have been discussed in detail (Hardy and DuBois, '38) and are considered here only as controls for the chill and exercise experiments.

In six of the experiments at 22.8°C. to 25.4°C., the naked men became so cold that they shivered. Five of these chills occurred during the experimental periods; one came on just after the observations had ended. In two other experiments within this temperature range, voluntary exercise prevented chills. Voluntary exercise was also studied in four experiments at temperatures higher than 25.4°C. in which there was no tendency toward chill during the period of the experiment. The data for the chill experiments are given in table 1 and for the exercise experiments in table 2. The experiments are listed according to increasing calorimeter temperature. In the first chill experiment at 22.8°C. subject D was not able to remain quiet for a full hour, and after 42 minutes, upon warning from the subject, the basal period was closed and the chill period begun. The experimental routine was such that periods shorter than 20 minutes could not be made although the chills quite often lasted only a few minutes. Therefore the actual increase in heat production during the chill itself could not be measured, and the heat production during the chill periods is the average production over the time of the period which includes the chill and several minutes of quiet.

In figure 1 are shown six typical experiments chosen from tables 1 and 2. Of the experiments included in the chart, two are chill experiments, two are of exercise in the chill range of temperature, and two are of exercise in the more normal, warmer atmospheres. All the relevant data are represented diagrammatically. The changes in rectal and skin temperature are plotted in the upper portion of the chart. The heat production in calories per hour is represented by the solid circles with lines through them; heat elimination, also in calories per hour, is plotted as a vertical column the width of which indicates the length of the period. Radiation loss R, convection loss C, and vaporization loss V are shown as divisions of the heat loss columns. The first chill experiment

TABLE 1
*Calorimeter data for chill experiments*

TABLE 1

*Calorimeter data for chill experiments*

| SUBJECT, DATE WEIGHT, REMARKS | END OF PERIOD | $CO_2$ | $O_2$ | R.Q. | $H_2O$ | URINARY N PER HOUR | PULSE RATE | TOTAL HEAT PRODUCED | CALORIMETER TEMPERATURE | CALORIMETER HUMIDITY | RECTAL TEMPERATURE | TOTAL HEAT ELIMINATED | SKIN TEMPERATURE | | RADIATION | CONVECTION | VAPORISATION |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | TIME | TEMP. | | | |
| | | *gm.* | *gm.* | | *gm.* | | | *cals.* | | % | | *cals.* | | | % | % | % |
| EFDB. Mar. 3, 1936, 76.9 kg. | 11.05 | | | | | | | | | | 37.15 | | 10.15 | 31.8 | | | |
| 1st period basal; 2nd period | 11.47 | 16.1 | 13.3 | 0.88 | 23.9 | | 53 | 44.8 | 22.81 | 29 | 36.86 | 73.0 | 10.40 | 30.6 | 58 | 23 | 19 |
| shivering; 3rd period cold; | 12.12 | 14.2 | 14.6 | 0.71 | 17.9 | | 50 | 47.6 | 22.86 | 32 | 36.75 | 47.3 | 11.52 | 29.8 | 49 | 29 | 22 |
| shivering in legs | 12.42 | 17.3 | 15.8 | 0.80 | 20.5 | 0.55 | 47 | 52.6 | 22.93 | 33 | 36.72 | 49.2 | 12.04 | 30.4 | 63 | 13 | 24 |
| | | | | | | | | | | | | | 12.47 | 30.7 | | | |
| JDH. Mar. 16, 1937, 67.4 kg. | 11.00 | | | | | | | | | | 36.84 | | 9.57 | 33.5 | | | |
| 1st period basal; 2nd period | 12.00 | 22.4 | 18.9 | 0.86 | 26.2 | | 53 | 63.2 | 22.88 | 30 | 36.66 | 87.3 | 10.50 | 32.4 | 73 | 9 | 18 |
| spontaneous chill from cold; | 12.30 | 27.7 | 25.2 | 0.80 | 19.5 | | 52 | 83.7 | 22.85 | 30 | 36.80 | 58.0 | 12.13 | 31.2 | 60 | 20 | 20 |
| 3rd period quiet | 1.16 | 16.4 | 12.1 | 0.95 | 18.6 | 0.75 | 54 | 41.1 | 22.75 | 30 | 36.88 | 59.1 | 12.27 | 30.7 | 72 | 10 | 18 |
| | | | | | | | | | | | | | 12.49 | 32.0 | | | |
| | | | | | | | | | | | | | 1.32 | 31.0 | | | |
| EFDB. May 12, 1936, 77.0 kg. | 11.00 | | | | | | | | | | 36.89 | | 10.39 | 31.2 | | | |
| 1st period basal; 2nd period | 12.00 | 22.4 | 19.3 | 0.84 | 31.2 | | 53 | 64.6 | 23.41 | 28 | 36.55 | 84.3 | 12.03 | 30.7 | 72 | 6 | 22 |
| spontaneous chill; 3rd period | 12.35 | 23.3 | 22.6 | 0.75 | 21.9 | | 49 | 74.4 | 23.53 | 28 | 36.48 | 58.3 | 12.13 | 31.0 | 59 | 19 | 22 |
| quiet | 1.28 | 22.4 | 19.8 | 0.82 | 27.2 | 0.55 | 47 | 65.8 | 23.56 | 28 | 36.66 | 82.2 | 12.39 | 31.0 | 73 | 8 | 19 |
| | | | | | | | | | | | | | 12.59 | 30.7 | | | |
| | | | | | | | | | | | | | 1.40 | 30.9 | | | |
| EFDB. Mar. 8, 1935, 75.8 kg. | 11.48 | | | | | | | | | | 37.27 | | 11.30 | 32.4 | | | |
| 1st period basal; 2nd period | 12.48 | 22.5 | 20.1 | 0.82 | 28.1 | 0.56 | 49 | 66.6 | 24.20 | 23 | 37.04 | 83.6 | 11.52 | 31.6 | 70 | 11 | 19 |
| shivering | 1.48 | 28.3 | 25.7 | 0.80 | 33.5 | 0.67 | 48 | 85.0 | 24.25 | 24 | 36.77 | 90.2 | 12.50 | 31.0 | 59 | 19 | 22 |
| | | | | | | | | | | | | | 1.38 | 30.7 | | | |
| EFDB. Feb. 11, 1936, 77.4 kg. | | | | | | | | | | | | | | | | | |
| 1st period basal, nearly | 11.35 | | | | | | | | | | 37.09 | | 11.06 | 32.7 | | | |
| shivering; 2nd period proba- | 12.35 | 23.7 | 21.6 | 0.80 | 32.3 | | 54 | 71.3 | 25.05 | 27 | 36.86 | 94.1 | 12.38 | 31.7 | 65 | 14 | 21 |
| ble muscle tensing; shivering | 1.13 | 15.3 | 14.9 | 0.75 | 22.0 | 0.68 | 50 | 48.5 | 24.98 | 28 | 36.71 | 60.5 | 1.17 | 31.8 | 50 | 29 | 21 |

TABLE 2

*Calorimeter data for exercise experiments*

| SUBJECT, DATE WEIGHT, REMARKS | END OF PERIOD | $CO_2$ | $O_2$ | R.Q. | $H_2O$ | URINARY N PER HOUR | PULSE RATE | TOTAL HEAT PRODUCED | CALORIMETER TEMPERATURE | CALORIMETER HUMIDITY | RECTAL TEMPERATURE | TOTAL HEAT ELIMINATED | SKIN TEMPERATURE | | RADIATION | CONVECTION | VAPORIZATION |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | TIME. | TEMP. | | | |
| | | *gm.* | *gm.* | | *gm.* | | | *cals.* | | % | | *cals.* | | | % | % | % |
| EFDB. Mar. 6, 1936, 75.9 kg. | 11.34 | | | | | | | | | | 37.19 | | 11.18 | 32.5 | | | |
| 1st period basal; 2nd period | 12.34 | 22.3 | 19.1 | 0.85 | 35.2 | | 51 | 64.0 | 25.01 | 31 | 37.05 | 85.1 | 12.38 | 31.8 | 67 | 9 | 24 |
| exercise; 3rd period quiet | 1.34 | 41.0 | 37.7 | 0.79 | 42.0 | 0.53 | 60 | 125.3 | 25.04 | 31 | 37.09 | 105.9 | 1.00 | 31.9 | 53 | 24 | 23 |
| | 2.34 | 23.8 | 20.1 | 0.86 | 35.2 | 0.55 | 48 | 67.6 | 25.12 | 31 | 37.16 | 90.4 | 1.15 | 32.1 | 62 | 16 | 22 |
| | | | | | | | | | | | | | 1.37 | 31.9 | | | |
| | | | | | | | | | | | | | 2.37 | 31.7 | | | |
| JDH. Mar. 2, 1937, 67.3 kg. | 11.00 | | | | | | | | | | 36.72 | 62.6 | 10.38 | 33.4 | | | |
| 1st period basal; 2nd period | 11.45 | 15.9 | 14.1 | 0.82 | 18.2 | | 58 | 46.9 | 25.12 | 22 | 36.64 | 71.7 | 11.55 | 32.3 | 68 | 15 | 17 |
| cold, exercised; 3rd period | 12.30 | 33.1 | 26.3 | 0.92 | 28.6 | | 72 | 90.1 | 25.10 | 24 | 36.72 | 83.0 | 12.46 | 32.7 | 57 | 20 | 23 |
| quiet, cold at end | 1.30 | 23.2 | 20.9 | 0.81 | 29.8 | 0.63 | 58 | 69.1 | 25.06 | 25 | 36.72 | | 1.49 | 32.2 | 65 | 17 | 18 |
| EFDB. Mar. 1, 1935, 75.8 kg. | 11.34 | | | | | | | | | | 37.30 | | 11.18 | 32.6 | | | |
| 1st period basal; 2nd period | 12.34 | 24.0 | 21.3 | 0.82 | 30.0 | | 53 | 70.9 | 25.85 | 24 | 37.20 | 81.7 | 12.36 | 32.1 | 66 | 13 | 21 |
| exercise | 1.34 | 36.8 | 33.8 | 0.79 | 39.6 | 0.47 | 58 | 113.5 | 25.87 | 27 | 37.18 | 98.4 | 1.36 | 32.4 | 55 | 21 | 24 |
| EFDB. Feb. 15, 1935, 74.9 kg. | 11.54 | | | | | | | | | | 37.38 | | 11.34 | 32.4 | | | |
| 1st period basal; 2nd period | 12.54 | 22.5 | 20.1 | 0.82 | 35.9 | | 54 | 66.7 | 27.00 | 25 | 37.21 | 76.4 | 1.09 | 32.6 | 60 | 13 | 27 |
| moving; 3rd period exercised | 1.29 | 15.6 | 14.8 | 0.77 | 23.4 | 0.51 | 50 | 48.7 | 27.05 | 26 | 37.17 | 49.2 | | | | | |
| by tensing muscles | 2.29 | 29.0 | 24.9 | 0.85 | 41.3 | 0.64 | 58 | 83.4 | 27.22 | 28 | 37.14 | 84.9 | 2.35 | 32.9 | 57 | 15 | 28 |
| JDH. Feb. 25, 1937, 66.4 kg. | 11.00 | | | | | | | | | | 36.84 | | 10.37 | 34.2 | | | |
| 1st period basal; 2nd period | 12.00 | 20.0 | 16.8 | 0.86 | 29.8 | | 56 | 56.6 | 28.00 | 21 | 36.68 | 64.7 | 12.07 | 33.5 | 65 | 9 | 26 |
| exercise; 3rd period exercise; | 12.30 | 18.1 | 13.8 | 0.95 | 20.7 | | 72 | 48.0 | 27.89 | 25 | 36.75 | 45.1 | 12.40 | 33.5 | 47 | 26 | 27 |
| 4th period exercise | 1.05 | 27.9 | 22.8[1] | 0.89 | 42.3 | | 78 | 77.8 | 28.07 | 37 | 37.10 | 65.3 | 1.12 | 33.5 | 37 | 25 | 38 |
| | 1.40 | 22.0 | 19.1 | 0.84 | 42.7 | 0.41 | 72 | 64.2 | 28.04 | 47 | 37.14 | 64.9 | 1.50 | 33.6 | 37 | 25 | 38 |
| EFDB. Feb. 18, 1936, 77.7 kg. | | | | | | | | | | | | | 10.17 | 34.6 | | | |
| 1st period basal; 2nd period | 10.34 | | | | | | | | | | 37.15 | | 11.40 | 34.4 | 57 | 13 | 30 |
| exercise; 3rd period exercise | 11.34 | 23.1 | 19.9 | 0.85 | 39.0 | | 57 | 66.4 | 29.81 | 27 | 37.02 | 72.3 | 12.10 | 34.5 | 47 | 19 | 34 |
| | 12.04 | 17.5 | 15.4 | 0.83 | 24.4 | 0.56 | 54 | 51.6 | 29.91 | 28 | 37.03 | 41.7 | 12.38 | 33.9 | 33 | 23 | 44 |
| | 1.04 | 41.1 | 36.8 | 0.81 | 85.3 | 0.65 | 59 | 122.9 | 30.12 | 39 | 37.17 | 113.2 | 12.54 | 34.4 | | | |

[1] Estimated.

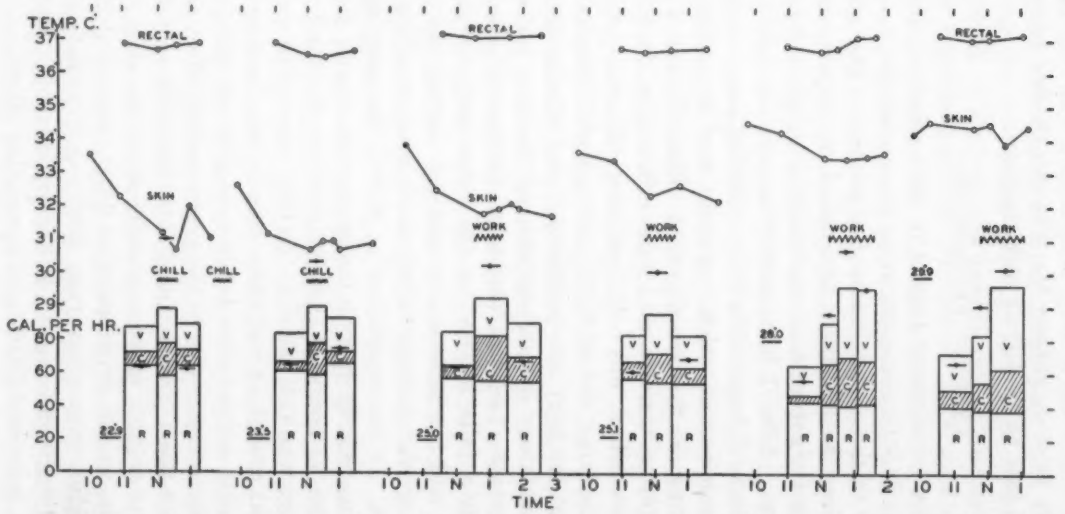


**Fig. 1** The effect of spontaneous chills and voluntary exercise upon the metabolism and thermal relations in two nude subjects. Columns represent heat loss in calories per hour. R, radiation; C, convection; V, vaporization. Heat production is shown by solid black dots with short horizontal lines. Calorimeter temperatures are given in figures just to the left of the columns.

and the second and third work experiments were on subject H; the others were on subject D. The calorimeter temperature is noted just to the left of each diagram.

### EXERCISE EXPERIMENTS

The exercise experiments (fig. 1) in the warm environment at 28.0°C. and 29.9°C. were performed in an atmosphere somewhat similar to the climate under our clothing. The rate of exercise during the last three periods of the experiment at 28.0°C. was not vigorous and consisted mainly of moving the arms and legs. After 1 hour and 40 minutes of such work, the total storage of heat in the body resulting from the exercise was 16 calories, of which 8 calories went to replace the heat lost during the first basal period. The net gain of heat by the body after exercise, at a rate sufficient to double the basal heat production, was only 8 calories; this was reflected by a relatively large rise of 0.3°C. in the rectal temperature. As rapidly as the heat was produced it was eliminated; the heat loss regulatory system endeavored to keep an exact balance between the heat production and the heat elimination. This regulation falls largely on the sweat and vasomotor mechanisms, and the increases in heat loss during the exercise periods are due entirely to increases in vaporization and to convection. In the experiment at 29.9°C. the stepping up of the heat loss to keep up with the production is well demonstrated. After 1½ hours of exercise at twice the basal rate, the net storage was 11.9 calories. In this experiment an outbreak of sweat caused a precipitate drop in skin temperature. This fall in skin temperature is characteristic of exercise at this temperature and is due to the great cooling effect of the sweat. Under these circumstances skin temperature reflects in no way the amount of vasoconstriction in the skin. The sweating rate, in the experiment at 28°C., increased 250% during exercise, convection rose 500%, and radiation remained practically unchanged. In the experiment at 29.9°C., vaporization increased 122% and convection 300%.

The exercise in the experiments at the colder temperatures, 25°C. and 25.1°C., prevented the subject from having a chill. During the first basal hour the body lost more heat than it produced. This loss was largely due to increased radiation, since vaporization and convection were approximately the same as in the warmer experiments. As soon as exercise was begun the body stored heat, the heat production went up about 100%, and the heat eliminated increased only 10% to 20%. Here again the increase in heat loss was due to vaporization and convection. In these two experiments shown in figure 1, vaporization increased 20% and 55% with exercise and convection increased 300%. The storage of heat in the body is reflected in both the skin and rectal temperatures. The last hours of the experiments were quiet periods with the heat production returning almost to the basal level and the body again losing heat rapidly.

In the exercise experiments, either in the warm or cold environments, the heat elimination rose relatively little until the body had restored the heat lost during the preceding period. The exercise did not last long enough in the colder experiments to produce a positive heat balance, but in the warmer experiments the negative balance was quickly removed and the heat elimination promptly began to adjust itself to the heat production.

At a calorimeter temperature of 27°C. subject D performed muscular exercise by remaining as motionless as possible while tensing the muscles—a very exhausting type of work. The idea was to observe what happened when there was increased heat production with no increase in heat loss by convection. The subject attempted to double his metabolism by fully extending the extremities and tightening the flexor and extensor muscles at the same time. The extremities were held in this extended position as motionless as possible. The subject did this first with one arm, next with the other arm, and then with both legs. It was very tiring and he thought he was increasing his heat production two- or threefold, a figure that he had attained in previous experiments flexing arms

and legs with less exertion. He was surprised and not a little annoyed when the observers outside the calorimeter kept urging him to work harder. As can be seen from table 2 he increased his metabolism only 25% and his heat elimination only 11%. Later it was realized that this exertion accomplished little work in the physical sense as there was no movement through space. The rise in metabolism observed is almost the maximum that can be obtained by this subject through this form of work and is very much smaller than that accompanying the mild exercise of the experiment just discussed. It will be noticed that the small amount of movement occurring during the second period of the experiment, due to voiding and measuring surface temperature, caused as great an increase in heat production as the exhausting muscular tensing in the last period. In spite of the subject's efforts to increase heat production without bodily movement, the convection loss increased 3 calories or 25%. Vaporization increased 3 calories or 13%, and radiation 2.5 calories or 5%. There was no appreciable storage of heat in the body during the exercise and the net bodily loss was 10 calories during the experiment.

### CHILL EXPERIMENTS

When a man undresses in an atomsphere of 25°C., slightly warmer than ordinary room temperature, and lies naked and motionless there is a striking drop in the temperature of the skin and subcutaneous tissues. Average surface readings may fall after 2 hours from an original level of 33°C. to as low as 30°C. The rectal temperature drops but little, 0.2°C. to 0.5°C. The man begins to feel cool, then cold, then chilly with an involuntary tensing of the muscles. At this time the metabolism rises slightly above the basal level. Then there comes a moment when the muscles begin to contract violently, the arms and legs jerk back and forth, and the whole body shivers perhaps so violently that the calorimeter shakes. The metabolism increases two- or threefold, but if the chill occupies only 5 or 10 minutes of a 30- to 50-minute experimental period the peak effect is obscured by general average.

The onset of the chill is almost a pleasant change from the effort to keep quiet and the man begins to feel more comfortable as he warms up his body. In these experiments it only took a short chill or moderate work to bring about a sense of comparative well-being. As a rule the chill ends abruptly and the man feels so comportable that he is able to stay quiet for an hour before there are symptoms of the approach of another chill.

In figure 1 are shown two typical chill experiments selected from the five given in table 1. The basal control period when the naked man is quiet shows the rapid rate at which the body is losing heat. The heat lost by radiation alone is almost equal to the heat production, and the convection and vaporization losses are net heat losses from the body. The skin temperature falls very rapidly until onset of the chill, and the rectal temperature drops at a rate not greatly different from that occurring at higher temperatures. Water loss from skin and lungs is also at approximately the same rate as at higher temperatures. The rapid fall in skin temperature is the only outstanding feature of the control period and this indicates that most of the body heat loss is occurring at the periphery.

With the onset of chill, the heat production jumps far above the heat elimination, averaging more than 100% above the basal level for 30 or 40 minutes. It is interesting to note that with all the body movement accompanying chilling, the heat elimination increases only 10% to 15% so there is considerable storage of heat in the body. The rises in skin temperature observed immediately after the chill are probably associated with the muscular need for oxygen producing a temporary vasodilatation. This causes a slight increase in heat loss due to radiation. The small increases in heat loss during periods of great muscular activity are characteristic of the experiments in the cold zone in which the body has been in negative heat balance, and are in sharp contrast with the prompt and large increases in heat loss in warm atmospheres.

During the period of the chill itself the heat loss by radiation is decreased because of low skin temperature. Convection loss increases about twofold and accounts for most of the 10% increase in total heat loss. Vaporization also increases from about 10% to 20% in most experiments and to 50% in one experiment. This is probably due to the greater volume of air breathed and possibly to an increased seepage of moisture through the skin accompanying the slight vasodilatation.

The chill cycle in both subjects was essentially the same, the body temperature seemingly maintained by a series of chills. The main difference in the chill experiments is the length of time of exposure required at different temperatures to bring on a chill. Obviously the colder the environment the shorter the exposure necessary to cause chill. This matter will be discussed more fully in a later publication.

As has been pointed out, the basal metabolism remained level at temperatures of 23°C. to 25°C., until a few minutes before the violent shaking of the chill even though the men were miserably cold. Rubner ('02) has described a 'chemical regulation' of metablism which is supposed to maintain heat production in the zone colder than that in which 'physical regulation' is sufficient. Martin ('30) and Cannon and his colleagues ('26) have advanced evidence to support this theory. If there were a 'chemical regulation,' one would expect evidence of it in the periods just preceding the chills. The complete absence of such evidence leads us to doubt the existence of Rubner's 'chemical regulation' in these two carefully studied normal men. This is in agreement with the work of Benedict ('15) and Swift ('32) who also found no evidence of a 'chemical regulation.'

In general it can be said that a chill occurs when the body becomes so cold that there is an urgent call upon the heat regulating mechanism. The exact site and location of the stimulus is still problematical. There are differences between individuals, and there must be many factors such as previous

exercise, state of health, sensory effects due to cold air currents, etc. Future investigations of this interesting phenomenon should include careful measurements of heat loss and its components as well as the heat production.

Chills which occur in disease with a resulting fever are similar in many respects to the chills occurring in normal persons as a result of cold. The point is brought out in figure 2 which a redrawing of an old chart published by Barr and DuBois ('18). The patient, George S., was studied in the Sage calorimeter, before, during and after a malarial chill. At that time the radiometer was not available and no partition could be made between radiation and convection. The heat production and heat loss were measured as well as the rectal temperature and vaporization. The patient was clothed in pyjamas and was comfortable in the calorimeter at 23.9°C. during the first two periods. The heat production in the two periods preliminary to the chill was above basal. The heat elimination was constant at about 10 calories less than the heat production. With onset of the chill the metabolism rose to a higher value than that occurring during spontaneous chill from exposure to cold in our experiments, and as the heat elimination rose only a very little a large amount of heat was stored in the body. The response of this patient to a rise in heat production differed from that of the normal subjects. The patient having a malarial chill stored all the extra heat in his body and retained it for several hours. In contrast, the normal man on producing extra heat eliminated it promptly.

The fever of the type of malarial chill, however, can be attained in the normal body if exercise be sudden and severe. This is shown in figure 3. The normal control, J.D.H., played squash racquets with one of the interns, Dr. C.W. Both were good players, evenly matched, and they played almost to the point of exhaustion. The radiating wall temperature of the squash court averaged 21°C., the air temperature 20.5°C. Before the start the men were weighed carefully on silk scales, accurate to 5 gm., and rectal temperatures measured with calibrated mercurial thermometers. The skin temperature and body radiation were measured in twenty different

places on the body surface. Dressed only in light running trunks they played squash violently for 12 minutes. They were weighed and temperature measurements repeated. This was done after three 12-minute exercise periods in rapid succession. Then they rested quietly in a dressing room when the

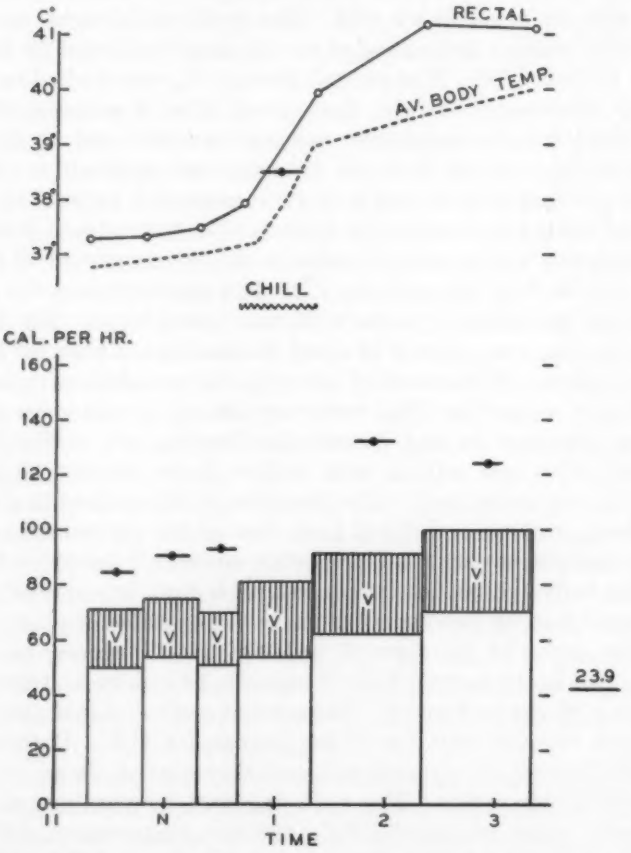


Fig. 2 Malarial chill observed in calorimeter (Barr and DuBois, '18). The average body temperature changes were estimated by subtracting heat loss from heat production.

measurements were repeated twice more. Radiation, of course, was determined accurately. Vaporization was estimated from approximate weight loss. Both men were sweating profusely and some dripping of sweat was observed, an error of 5% to 10% may have been made in estimating this

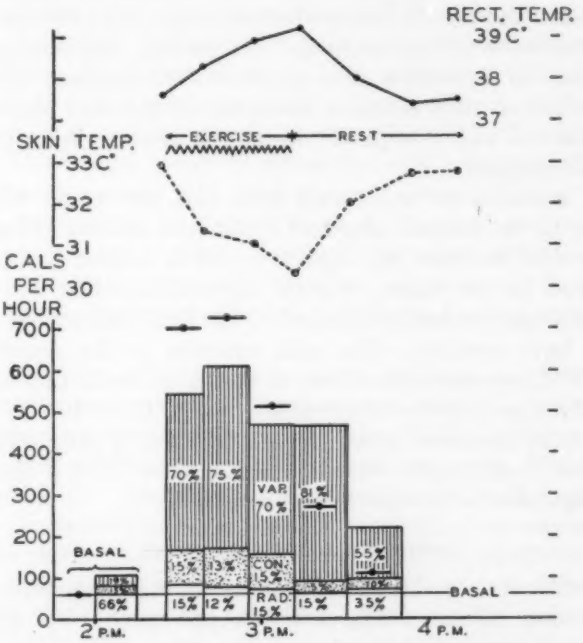


Fig. 3 Effect of violent exercise upon metabolism and heat loss.

dripping. Convection was estimated from other work experiments in the calorimeter and is only approximate. Heat elimination was the total of radiation, convection and vaporization. Heat production was estimated from elimination and heat storage. It also is only approximate. Skin temperatures could not be used in this experiment to estimate storage of heat in the body.

The main point is the rise of rectal temperature in J.D.H. to 39.2°C. (102.6°F.) and in C.W. to 39.3°C. (102.7°F.). Just as striking is the fall in average surface temperature in spite of the fact that within 1 minute of the onset of exercise their skins became pink from the increased blood flow. When the exercise stopped the rectal temperature fell to normal within 25 minutes and the surface temperature rose to its former level when visible sweating stopped. The normal bodies could not take care of a sudden ten- to twelvefold increase of heat production so they attained fever levels but they increased vaporization and dissipated the stored heat as soon as the exercise stopped.

The malarial patient would have lost his stored heat as rapidly as the normal men had it not been for the change of the level of his heat loss regulator. It is evident that if the heat loss in the warm exercise experiments had not been carefully and promptly adjusted to the heat production, fever would have resulted. The mild exercise in the experiment at 29.9°C., assuming heat loss at the basal level, would have caused a rise in body temperature to 38.1°C. or 101°F. The extra heat produced after meals from the specific dynamic action of food is quite sufficient to produce a fever if the heat loss regulator is not operating properly.

## SUMMARY AND CONCLUSIONS

Two naked normal men were studied in the Sage respiration calorimeter at temperatures which produced chills after 2 to 3 hours exposure. Six observations were made on involuntary chills and six on voluntary exercise. There was no essential difference between chills and exercise in regard to radiation, convection, vaporization, surface temperature and rectal temperature. When chills were imminent voluntary exercise would check them.

When naked men lie motionless in a calorimeter at 22°C. to 25°C. the rectal temperature falls 0.2°C. to 0.5°C. in 2 or 3 hours, but the average surface temperature falls about 3°C. and the man shivers. This exercise usually but not

always causes a slight rise in average surface temperature and warms him enough to stave off a second chill for an hour or so. The shivering may store 20 to 40 calories in the body.

At temperatures about 27°C. when the men are comfortable, exercise causes a marked increase in the percentage of heat loss by vaporization. At lower temperatures chills and exercise cause little or no change in this percentage. The total amount of radiation diminishes slightly on account of the falling skin temperature, and the percentage of heat lost by radiation decreases markedly. Convection is greatly increased by the movement of the extremities in chills and exercise. This is one manner in which the body may give off more heat through a cool skin than through a warm skin.

The metabolism in these two men remained at its basal level until 5 to 10 minutes before the onset of the chills, casting doubt on Rubner's 'chemical regulation.' It fell to basal levels promptly after the chills and after moderate exercise.

Two athletes after playing squash racquets violently for 36 minutes showed rectal temperatures of 39°C. with falls in average surface temperature of about 3°C. After resting 40 minutes rectal and surface temperatures returned to their former levels. Radiation and convection were changed but little. Increased vaporization dissipated the large amount of heat stored in the body. Here by a different mechanism the body again gave off more heat through a cool skin than through the warmer and drier skin of the basal periods.

The chills of malaria are contrasted with the chills due to cold and with the fever due to hard exercise.

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# HEAT LOSS FROM THE NUDE BODY AND PERIPHERAL BLOOD FLOW AT TEMPERATURES OF 22°C. to 35°C. [1]

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TWO FIGURES

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In previous publications were described (Hardy and DuBois, '38 a, b; Hardy, Milhorat and DuBois, '38 a, b) experiments dealing with the effects of chills, exercise and exposure to different environmental temperatures on two nude subjects. No attempt was made to analyze these experiments in terms of the established laws of heat transfer, although it was tacitly assumed that these laws did hold. In recent years, Burton ('35), Burton and Bazett ('36) and Winslow, Herrington and Gagge ('36 a), have made excellent studies of the heat loss from the body from a physical standpoint, but in none of these works were the data as complete or as precise as in the calorimeter studies which have been published from the Russell Sage Institute of Pathology. It is the purpose of the present paper to discuss these data in a manner similar to that of the authors just mentioned and to give a direct experimental comparison between the heat loss in man under our 'standard' conditions with that from a similarly shaped physical body under the same conditions.

[1] Clinical Calorimetry Series, no. 53.

Heat is transferred from the musculature and viscera to the body surface whence it is dissipated into the environment by radiation, conduction, convection and vaporization. The physical factors of importance in this heat loss are the internal body temperature, the thermal gradients in the superficial tissues, the skin temperature, body movement, the heat production and the amount of water vaporized from the skin. The environmental factors are the air temperature and velocity, relative humidity and wall temperature. In the basal experiments the subjects lay motionless so that body movement may be considered zero. The internal body temperature (rectal temperature), the skin temperature, the heat loss and water vaporized were all measured. The gradients in the superficial tissues were not measured, and it is doubtful if a sufficient number of measurements of the type made by Bazett and McGlone ('27) could be made to be of service in this work. Unquestionably the internal gradients on the forehead and trunk are quite different from those in the arms and hands and feet. As was pointed out in a previous paper, the mass of tissue lying within 1 cm. of the body surface is multiplied by the skin temperature and added to the remainder of the body mass multiplied by the rectal temperature in order to arrive at the average body temperature. This would mean that the average gradient over the whole body would extend an average of 20 mm. below the skin. This figure is in approximate agreement with the values found by Bazett and McGlone and is assumed to be the average depth below the skin surface necessary to go before reaching a temperature representative of the internal body temperature. Limitations of this assumption are recognized and they will be discussed. Of the environmental factors only one was not measured, the air velocity, and this was so small that even with the ventilating pump of the calorimeter running at maximum speed there were no perceptible currents of air over the surface of the naked subjects. For this reason the air is considered as still air, with currents due mostly to the natural convection from the warm surface of the body.

The experimental data necessary to the discussion of the basal experiments are plotted in figure 1.

The topmost curve is the rectal temperature of the two subjects; the second curve is the average skin temperature; the third curve, the average heat loss curve is compared with the basal heat production. The heat production in both these

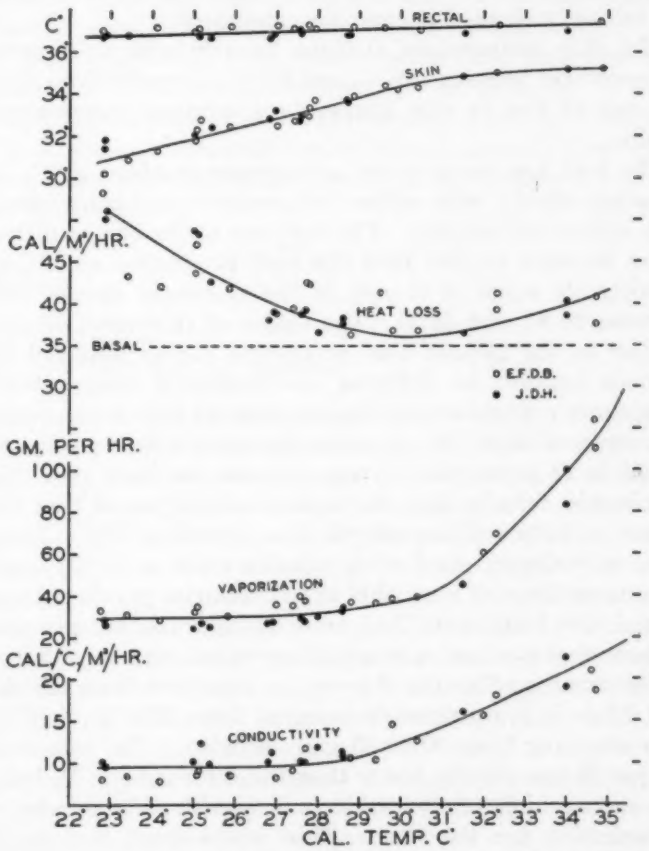


Fig. 1 Relationship of factors involved in heat loss to environmental temperature.

subjects was 34.9 cals./m.²/hour, and did not vary appreciably from 22°C. to 35°C. during the first few hours of the experimental period. The vaporization rate is shown in the next curve, plotted in grams per hour.

The rectal temperature level decreases uniformly, about 0.2°C. for each degree fall in calorimeter temperature. The rectal temperature here plotted was taken about 2 hours after the subject undressed to enter the calorimeter.

The skin temperature changes linearly with calorimeter temperatures between 22.8°C. and 30°C. From 30°C. to 35°C. the rate of rise in skin temperature becomes progressively smaller.

The heat loss curve shows a minimum at about 31°C., increasing rapidly with colder temperature and more slowly with higher temperature. The heat loss under our conditions never becomes smaller than the heat production and is approximately equal to it only in the relatively narrow zone between 29°C. and 32°C. The shape of this curve is very similar to the 24-hour heat production curves obtained for animals exposed to different environmental temperatures. This curve represents also the minimum 24 hour heat production curve of these two nude men because the heat production would in 24 hours have to approximate the heat loss. The men would actually have to produce slightly more than this as any activity will increase the heat loss 10 to 15%. Therefore, an ordinary sized man, exposed nude at 23°C., would have to produce at least 2300 to 2500 calories per day simply to maintain body heat. In 1 hour the approximate minimum 24-hour heat production at any temperature may be obtained.

The number of grams of moisture vaporized from the skin and lungs is approximately constant from 22.8°C. to 30°C., only changing from 30 to 33 gm. per hour. The values for subject H are slightly lower than those for subject D, but if the surface area of the subjects is considered the rates of vaporization are the same. This would mean that in the lower temperature range the water loss from the lungs is proportional to the surface area, because here the vaporization from the lungs is slightly more than half the total. The

fact that the water vaporized is constant and at a minimum level through this wide range of temperature indicates that the portion of water lost through the skin is due to seepage or transudation of water through the tissues. Thus, the amount of water vaporized would be proportional to the surface area of the body. The grams of moisture lost per unit of time must bear definite ratio to the heat production if the heat production is proportional to the surface area.

Above 30°C. the vaporization curve rises very rapidly to take care of the body cooling. From 23°C. to 30°C. the vaporization loss cares for a constant amount of heat and the changes in total heat loss are due to convection and radiation. One may set, in the usual way, as the transfer of heat to the periphery,

$$H = K(T_R - T_S) \times S \times t \qquad (1)$$

Where,

$H$ = heat loss
$K$ = conductivity
$T_R$ = rectal (internal) temperature
$T_S$ = skin temperature
$S$ = surface area
$t$ = time

All of the factors in this equation except K are measured directly so that K is determined. It is evident that the question of vaporization does not enter this equation directly so that K may be calculated throughout the experimental range (23°C. to 34°C.). This value is plotted at the bottom of figure 1 as the conductivity in calories per degree per square meter per hour. The value of K is independent of calorimeter temperature between 22.8°C. and 28°C. and has an average value of about 9.1 calories per degree per square meter per hour in this range. At 28°C., K begins to rise in proportion to the rise in calorimeter temperature. The conductivity may be considered as consisting of two parts: the convection of heat by means of the blood stream and the conduction of heat through the tissue itself. The vasomotor reactions of the body in response to heat and cold are well known and consist of decreasing the peripheral blood flow in

cold and increasing it in heat. Thus, one can write,

$$K = K_t + K_b$$

where

$K$ = observed conductivity,
$K_t$ = average conductivity of superficial tissue,

and

$K_b$ = average amount of blood flowing across a unit area.

The factor $K_t$ would be expected to be a constant depending only on the structure and composition of the tissue. $K_b$ would change with vasomotor activity, becoming maximum with vasodilatation and almost zero with vasoconstriction. Inspection of the conductivity curve shows that the curve is composed essentially of two parts: the part below 28°C. in which the conductivity is constant and minimal, and the part above 28°C. in which the conductivity changes almost linearly with temperature. According to the present interpretation $K_b$, or the blood flow, changes rapidly with temperature at temperatures higher than 28°C., and below 28°C. becomes so small as to be insignificant. This does not mean that vasoconstriction has ceased at this temperature but only that restriction of peripheral blood flow is no longer of any importance in heat transfer at this or any lower temperature. $K_t$ is thus represented quantitatively by the flat portion of the curve, and the heat is being carried by pure conduction through the tissues to the skin. In this circumstance the body tissue is acting like any other physical insulator and the laws of heat conduction apply directly. Lack of sufficient knowledge of the internal thermal gradients of the body prevents the direct application of these equation to any specific body area. However, one may estimate the average specific thermal conductivity of peripheral tissue, as Lefèvre ('11) and Burton ('35) have done, on the assumption that the average gradient extends in 20 mm. from the skin surface. This leads to the value

$K_t = 0.0005$ gm. cals./sec./cm.$^2$/°C./cm.

This value compared with leather (0.0004), paper (0.0003), and cork (0.0007) shows a high thermal insulating quality of tissue. Our direct experimental observations of the thermal conductivity of beef muscle and beef fat give the values 0.00047 and 0.00049 respectively. Thus the value 0.0005 for body tissue is based on the minimum depth of thermal gradient, that is, 20 mm. For values of conductance lower than 9.1 kg. cals./°C./m.²/hr., the average depth of the thermal gradient must be greater than 20 mm. It is obvious that the average distance below the surface necessary to go to reach rectal temperature will be greater for a fat person than for a thin person, but it is not proper to think that the fat person has any more insulation against cold by reason of this. From the observations in figure 1 on the conductance values of subjects H and D, one can estimate the depths of the thermal gradients as 18 mm. and 22 mm. respectively. It is evident that blood flow is not a significant factor here, because if one assumes that $K_t = 0$ and that all the heat in the range from 28°C. down were carried by the blood, the rate of flow of blood per cm.² of peripheral tissue would be 0.015 cc. per minute, or about 2 drops of blood every 10 minutes. If the tissue be the chief conductor in this region, as evidently it is, the flow of blood must be less than a tenth of this amount. Calculation of the oxygen need of resting muscle shows that this small amount of blood is adequate to supply the metabolic requirements.

From 28°C. up to 35°C. the blood flow increases linearly with temperature at the rate of 37 cc. per minute per square meter for each degree rise in temperature. At 35°C. the peripheral blood flow is about 0.23 liters per minute per square meter, or about 10% of the basal cardiac output. In severe exercise, as in the experiment discussed by Hardy, Milhorat and DuBois ('38 b), the peripheral circulation rose to 23 liters in 11 minutes or 1.2 liters per minute per square meter, about 55% of the resting cardiac output. However, the increased circulation was not sufficient to prevent a rise in body temperature.

The equation for the total conductivity may be rewritten, since $K_t$ is constant and $K_b$ varies uniformly with temperature above 28°C. Thus,

$$K = 0.00026 + 0.00006\ (T_s - 28)\ \text{gm.cals./sec./cm.}^2\text{/°C.}$$
$$= 9.1 + 2.2\ (T_s - 28)\ \text{kg.cals./m.}^2\text{/hour/°C.}$$

The last term of the equation is zero for $T_s$ less than 28°C.

It is interesting to compare the conductivity curve with the skin temperature curve in figure 1. Between 22.8°C. and 28°C. the actual change in peripheral blood flow is insignificant, although the skin temperature changes almost 4°C. From 28°C. to 35°C. the blood flow increases about threefold and the skin temperature rises only about 2°C. Thus average skin temperature alone says nothing in these circumstances about blood flow. The reasons for this are obvious.

In the range below 28°C. the fall in skin temperature is due entirely to the physical cooling of the body. The vasomotor action is insignificant in preserving body heat. The heat loss increases and the vaporization is constant; all these things are expected if the body is cooling like an inanimate object. It will be noticed that the conductivity curve starts rising at 28°C. and the vaporization curve at about 30°C. In this 2° range the body is controlling its heat loss through vasomotor action alone, and we have termed this the 'zone of vasomotor control' of heat loss. This is the low temperature half of the zone of neutrality in which the heat loss approximates the heat production. In this temperature range the subjects felt most comfortable, and it is probable that here the heat loss could be controlled without conscious effort on the part of the subjects. The average skin temperature in this zone is 33.8°C. Study of the skin temperature under the clothing of these subjects when exposed to various temperatures showed a plateau of skin temperature at 33.8°C. This may be considered as the physiological basis of what the air conditioning engineers call the 'comfort zone.' That is, the 'comfort zone' is probably the zone of vasomotor control of heat loss under resting conditions.

At temperatures higher than 30°C. the vaporization curve and the peripheral blood flow go up together, the skin temperature rising but slightly (0.7°C.) and the rectal temperature increasing very gradually (0.2°C.). The heat loss begins to rise in these two subjects, probably a slight overcooling by sweating. This zone is one of control of heat loss by the combined efforts of the sweating and vasomotor mechanisms. The heat loss regulation in this zone is very delicate indeed and is adjusted continually to the approximate needs of the body. A change in environmental temperature from 32°C. to 33°C. will cause a 33% increase in vaporization and a 37% increase in peripheral blood flow. The human body seems to have been provided with an excellent regulator of heat loss at temperatures higher than 28°C. It is worthy of note that although the cooling in these hot atmospheres was more than adequate to eliminate the body heat, the subjects were uncomfortable. The precise regulation of the rate of peripheral blood flow and sweat production is probably dependent upon this sensory effect. It seems that the regulation cannot depend upon rectal, or average body temperature, as these factors are changing but little. An attractive hypothesis is that the blood flow to the skin increases in response to a sensory stimulus and that the increased blood flow itself produces a sensory effect which increasing to a certain threshold causes the production of a certain amount of sweat. The increased sensory effect of warmer environments in turn produces more blood flow and more sweat.

In the colder atmospheres there seems to be no automatic control of heat loss, and the body responds to environmental temperature in the same way as does any physical object. This lower temperature range is really a zone of no internal control of heat loss. It was in this zone that a comparison between heat loss from a physical object and from the subjects was made. The previous discussion has had largely to do with the transfer of heat to the surface of the body; the comparison with the physical body will deal with the transfer of the heat into the environment, in the temperature range below 28°C.

An elliptical, galvanized iron cylinder, 50 cm. long and 18 cm. and 30 cm. in minor and major axes, was selected as having somewhat the size and shape of the trunk of a man and as having an area which was easily calculated and completely effective for heat loss. The outer surface of the cylinder was painted black with a paint whose emissivity on testing was found to be about 93%. The surface area of the cylinder was 0.48 sq.m. and its mass when filled with water was 24.98 kg. The cylinder and its contents were heated by means of a small direct current motor which ran submerged and at the same time furnished vigorous stirring for the water. The caloric input to the cylinder was measured with a carefully calibrated voltmeter and ammeter, the electrical energy being derived from a large 12 volt storage battery 'floating' on the 110 volt power line. The electrical energy could then be held constant within $\pm 0.02\%$. The temperature of the cylinder was determined to 0.01°C. with a precision resistance thermometer, and the surface temperature measured with a radiometer. The thermal capacity of the cylinder and contents was calculated from approximations of the known masses of different materials used in its construction to be 20.9 kg. calories per degree. This constant was also determined experimentally from data on heating and cooling. The data of twenty measurements resulted in the value $20.69 \pm 0.22$. The cylinder was mounted on four wooden insulating posts 4 inches from the floor of the respiration calorimeter. In this way the heat loss by conduction was rendered so small as to be insignificant and Newton's law of cooling in still air could be applied. The factor of thermal conduction was also eliminated from the studies on human subjects by having the subject suspended in a tight hammock of coarse fish line with a 4 cm. mesh. Newton's cooling law is:

$$H = C\,(T_s - T_c) \times t \times S$$

where

$H$ = heat lost
$C$ = constant
$T_s$ = the surface temperature
$T_c$ = the calorimeter temperature
$t$ = time
$S$ = surface area.

An example of the applicability of this formula to the cylinder and of the degree of agreement between the electrical heat put in and that measured by the calorimeter is given in table 1.

It is seen that Newton's law applies to the cylinder with an accuracy better than the agreement between the electric calorimeter and the respiration calorimeter. The disparity between the 'direct' and the electrical calorimetry was always less than 5% and generally less than 2.5%. The effect of the circulation of air through the calorimeter was tested by comparing the cooling constant with the circulating pump running, with that when it was shut off. The two values were the same, substantiating the assumption previously made that

TABLE 1

*Comparison of electrical and direct calorimetry*

| *Period* | *Electrical input* cals. | *Calorimeter heat* cals. | *Difference* % | *Newton's law constant* |
|---|---|---|---|---|
| 1 | 13.48 | 13.32 | −1.2 | 1.64 |
| 2 | 13.53 | 13.69 | +1.2 | 1.63 |
| 3 | 14.68 | 14.54 | −1.0 | 1.63 |
| 4 | 14.98 | 14.57 | −2.7 | 1.65 |

the calorimeter air was practically still even with the pump running. The position of the cylinder in the calorimeter was of great importance. Raising the can from its usual position 10 cm. from the calorimeter floor to a position 30 cm. from the floor caused the cooling constant to change from 1.64 to 1.72. This change was undoubtedly due to the increased convection loss in the elevated position. As the position of the human subjects was more nearly 5 cm. to 10 cm. from the calorimeter floor, the position of the cylinder at 10 cm. was considered as standard.

Winslow, Herrington and Gagge ('37) have found that of the purely thermal loss from their subjects, 51% and 55% was convection and 49% and 45% radiation. Their subjects were studied under conditions more favorable to convection than the present conditions under which convection amounts to

only 20% of the thermal loss and radiation 80%. Furthermore, their subjects were not basal or motionless so that larger convection losses would be expected. That the convection losses in the present work are of the right order may be seen from a comparison of the thermal loss of the subjects with that of the cylinder.

In order to compare the radiation and convection losses from the cylinder with those losses from the subjects, the vaporization factor is omitted. Vaporization, being practically constant from 28°C. down, is of importance only as an additive factor. Experiments under basal conditions, temperature 25°C. to 26°C., on the nude subjects showed that 66% to 68% of the total heat loss was radiation, 21% vaporization, 11% to 13% convection. Therefore, of the 79% of the total loss 80% was radiation and 20% convection. The cylinder was placed in the calorimeter at this temperature in the same position as that used for the men, and tests made of the radiation and convection losses. The values for radiation from the cylinder were from 72% to 74% and for convection 28% to 26%. If the radiation from the cylinder be increased by 7%, to correct for the emissivity of the paint, radiation becomes 78% and convection 22%. Thus, on a comparable basis, the proportions of heat lost through radiation and convection are the same for the cylinder and the subjects.

A comparison of the rates of cooling per unit area of skin and of cylinder surface is of interest. The cooling constants have been plotted for the men in figure 2 and the average value for C. is 5.3 calories per degree per square meter per hour, using the DuBois surface area. The surface area of the cylinder is easily estimated from its dimensions, and all of its area is effective in heat loss. The emissivity of the cylinder is 93% compared with about 99% for the men, so that, assuming the same convection loss in both cases, the men would be expected to cool about 6% faster than the cylinder. It must be remembered, however, that all the surface of the cylinder is effective in heat loss whereas a considerable portion of man's area is not. Thus, it was not surprising that

the cylinder gave a value of 6.75 calories per square meter per hour per degree which is considerably higher than that for the men. Comparing the rates of heat loss on a same

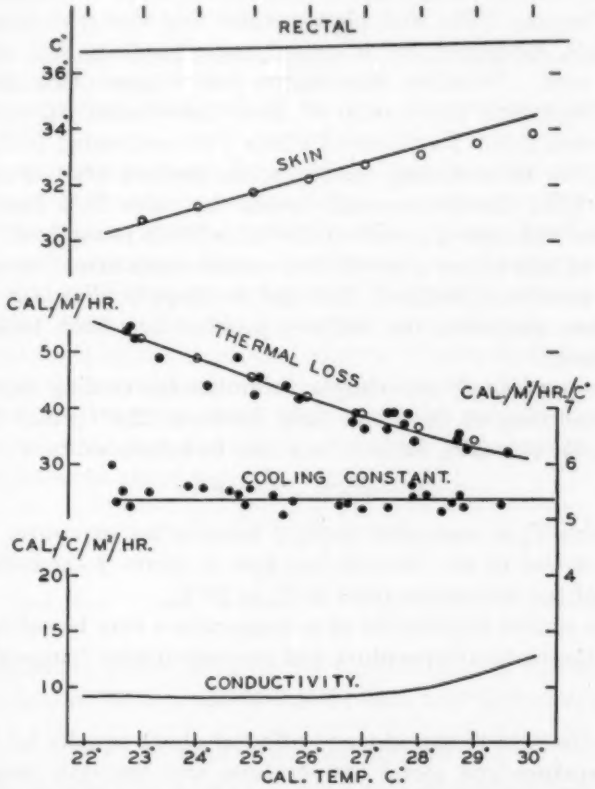


Fig. 2 Change in the thermal properties of the body with calorimeter temperature. Solid lines represent average values from measurements on subjects. Solid dots represent observed values; open circles calculated values.

basis gives a method of estimating the effective surface area of the subjects. Simple proportion of the radiation losses shows that 75% of the men's total surface is effective in heat loss when lying stretched out with arms close to the side and

feet together. This constant is one of the most difficult of the important factors in this study to estimate. Rubner ('02) first called attention to the fact that the 'effective' surface area in man is always less than the total area. Bohnenkamp and Pasquay ('32) with photographic and electrical capacity methods, estimated, for a spread eagle position, 85% of the total area. Winslow, Herrington and Gagge ('36) with a very ingenious application of their partitional calorimeter estimated 75%. Hardy and DuBois ('38) estimated 78%, and using the Bohnenkamp photographic method arrived at the value 77%. In the present studies the value 78% has been adopted although a possible error of $\pm 2\%$ is recognized. The value of this factor depends to a considerable extent upon the body position (Bedford, '35) and in these studies, for comparative purposes, the military position has been taken as standard.

One may apply the simple formulae for cooling rates to the reactions of the nude body between 22.8°C. and 28°C. Thus, for example, the heat loss may be calculated by

$$H = 4.2\ (T_R - T_0) \times S \times t$$

in which $T_R$ is measured about 2 hours after exposure. The open circles in the thermal loss line in figure 2 show the results of the calculation from 29°C. to 23°C.

In a similar manner the skin temperature may be calculated from the rectal temperature and the calorimeter temperature,

$$T_s = 0.44\ T_s + 0.56\ T_R.$$

Thus the skin temperature is affected about equally by room temperature and rectal temperature, and the skin temperature rises almost half a degree for each degree rise in room temperature. This was pointed out by Wiley and Newburgh ('31).

The relationship between the heat loss and the calorimeter and skin temperatures over the whole range of temperatures from 22.8°C. to 35°C. may be stated in the formula,

$$H = (9.1 + 2.2\,(T_c - 28))\,(T_R - T_s)$$

where

$H$ = heat loss in calories per square meter per hour
$T_c$ = calorimeter temperature, 28° or higher
$T_R$ = rectal temperature
$T_s$ = skin temperature.

The usefulness of such a formula is limited to the conditions under which it was derived and these include absolute quiet, naked body, low humidity (30% to 50%), no conduction loss and small convection loss. The formula is given mainly to show that very simple relationships hold for the thermal exchanges of the body, and as simple a relationship may be derived for a less restricted condition. Under circumstances in which the rectal temperature represents the internal body temperature and the heat loss and skin temperature are measured, the total flow of blood to the skin may be computed from the above formula. Thus,

$$F = 17\,S\,\left(\frac{H}{T_R - T_s} - 9.1\right)$$

where

$F$ = blood flow in cubic centimeters per minute
$S$ = total area of the body.

The extent to which this formula may be applied will depend upon the conditions under which the heat loss is measured.

## SUMMARY AND CONCLUSIONS

Data obtained on heat loss in the Russell Sage calorimeter have been analyzed as regards the transfer of heat from the interior of the body to the skin and from the skin into the environment at temperatures of 22°C. to 35°C.

The heat loss from a blackened cylinder similar in shape to a man's trunk was studied and a comparison made with the heat loss from the skin.

1. Attention is called to the shape of the heat loss curve when heat loss from the nude, motionless body is plotted as a function of environmental temperature. The curve is very similar to the 24-hour heat production curves obtained from animal experiments at different temperatures. Neutral temperature range for basal, nude humans is 28°C. to 31°C.
2. The reaction of the body to temperature, as regards heat loss is divided into three zones:
   A. Cold zone, temperatures below 28°C., no regulation of heat loss.
      In this temperature range the body cools as a physical body; vasomotor effects are insignificant as regards heat loss.
      Vaporization is constant and proportional to the surface area.
      Thermal conductivity of the peripheral tissues is constant and has a value of approximately 0.0005 gm. calories per second per cm.$^2$ per degree per centimeter.
      Blood flow to the skin is of the order of 0.015 cc. per minute per cm.$^2$ and is constant.
      The decrease in skin temperature with lowered environmental temperatures is the result of physical cooling and not of vasoconstriction.
   B. Zone of vasomotor regulation, 28°C. to 30°C.
      Heat loss regulated by changes in peripheral blood flow.
      No sweating.
      Subjects were most comfortable in this zone.
      Heat loss is minimum.
   C. Zone of vasometer and evaporative regulation, temperatures above 30°C.
      Blood flow to the skin increases with temperature.
      Vaporization increases but not in exact proportion to the temperature.

Surface temperature rises but little.
Cooling of the body is adequate, but subjects are not comfortable.
Heat loss rises slowly with temperature.

3. The average peripheral blood flow, as a function of heat loss, surface area, skin temperature, and rectal temperature, is determined for the particular experimental conditions.
4. The relationship of basal heat loss to skin, rectal and environmental temperatures is also calculated for these conditions.
5. Newton's cooling law applies precisely to human subjects, when motionless, at temperatures lower than 30°C.
6. Ratio of the rates of heat loss from the cylinder and the subjects shows that 75% of the subjects' total area is effective in losing heat by radiation and convection under our 'standard' conditions.

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